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SYNDROME CHARACTERIZED BY OSTEITIS FIBROSA DISSEMINATA, AREAS OF PIGMENTATION, AND A GONADAL DYSFUNCTION

FURTHER OBSERVATIONS INCLUDING THE REPORT OF TWO MORE CASES[1]

FULLER ALBRIGHT, BEECHER SCOVILLE
AND
HIRSH W. SULKOWITCH
From the Medical Service of the Massachusetts General Hospital and the Department of Medicine of Harvard University Medical School
BOSTON, MASSACHUSETTS

The syndrome which is the subject of this paper has recently been described in detail (1) and its literature reviewed.[2] It is the object of this paper to report two additional cases, and to discuss certain added information concerning the syndrome which they bring out. Briefly, the characteristics of the syndrome are as follows: *a)*. A disseminated osteitis fibrosa (both hyper and hypo-ostotic), with a distribution suggesting a relationship between the lesions and nerve roots or an embryologic defect in the myotomes; *b)*. Areas of cutaneous pigmentation which have a distribution suggesting some connection between them and the bone lesions; *c)*. Sexual and somatic precocity especially, if not exclusively, when the disease occurs in the female sex.

In the previous paper (1) an attempt was made to explain possible relationships between these apparently disconnected manifestations. It was thought that the distribution of the bone and cutaneous lesions was such as to preclude an endocrine or metabolic disturbance as their cause. On the other hand, the possibility of a neurologic (or possibly embryologic) defect seemed highly possible. The question was then discussed whether the gonadal disturbance might not be due to a disturbance in the afferent impulses to the anterior pituitary, thus perhaps explaining the whole bizarre syndrome on one basis—a disseminated neurologic lesion.

[1] Read before the 21st Annual Meeting of The Association for the Study of Internal Secretions, Atlantic City, N.J., June 8, 1937.

[2] Since this paper was read, still another case has been studied at the Massachusetts General Hospital and will be reported elsewhere. This case has previously been published (Hirsch, I. S.: Radiology 13:44. 1929) but was missed in the review of the literature in the first communication (1).

CASE REPORTS

Case 6. A. J. K. (M.G.H. No. 26700), a white male aged 21 years, was sent to the Massachusetts General Hospital from the Hartford Hospital by Dr. Arthur D. Baldwin in Feb. 1937, with the tentative diagnosis of hyperparathyroidism. At the age of 4 or 5, it was noted that his right leg began to bend below the knee, in consequence of which an osteotomy was performed. At the age of 8, as the result of very slight trauma he sustained a lesser trochanteric fracture through the right femur.

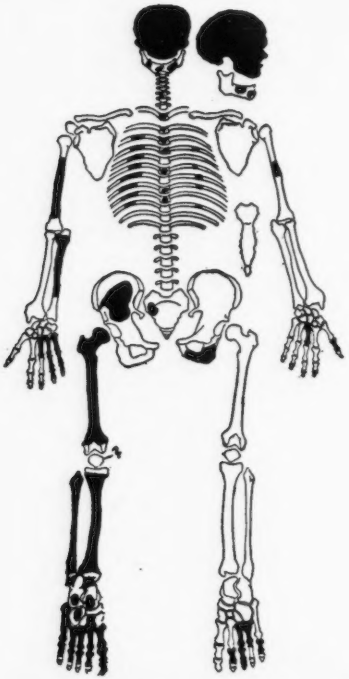

Fig. 1. Diagram to show distribution of skeletal lesions in *Case 6*. The lesions except for those in the skull were predominantly right-sided, corresponding with the areas of pigmentation (fig. 2).

A roentgenogram at that time showed antero-external bowing of the right femur with coxa vara and cystic degeneration in the mid-femoral neck. At the age of 15, the right arm was fractured while fighting with another boy.

At the age of 18, he again broke his right femur as the result of a fall while chasing a rabbit. His serum calcium level was found to be 12.4 mg. per 100 cc., and his serum inorganic phosphorus level 2.9 mg. per 100 cc. Roentgenograms showed multiple cystic areas. A diagnosis of hyperparathyroidism was made, but no parathyroid tumor was found at operation by Dr. A. M. Rowley. A subtotal thyroidectomy was performed, and one normal parathyroid was removed. Three months later, his serum calcium level was found to be 11.9 mg., and his serum phosphorus 3.6 mg. per 100 cc.

In February 1936, he entered the Hartford Hospital for his fifth admission with

Fig. 2. *Case 6.* Note segmental distribution of areas of pigmentation.

Fig. 3. *Case 6. A.* Roentgenogram of pelvis. Note complete absence of involvement of left side of pelvis, except for lesion in ischium; increase in size and density of involved left ischium; large circumscribed cyst in right ileum and marked cystic degeneration of whole upper end of left femur.

B. Roentgenogram of feet. Note cysts in shafts of metatarsals.

C. Roentgenogram of right tibia and fibula. Note characteristic homogeneous texture of bone.

a transverse fracture through his right tibia, as the result of stubbing his toe. The calcium and phosphorus levels in the serum were now normal, 10.8 and 3.3 mg. per 100 cc., respectively. In December 1936, he sustained multiple fractures on both sides of the body as the result of severe trauma resulting from an automobile accident.

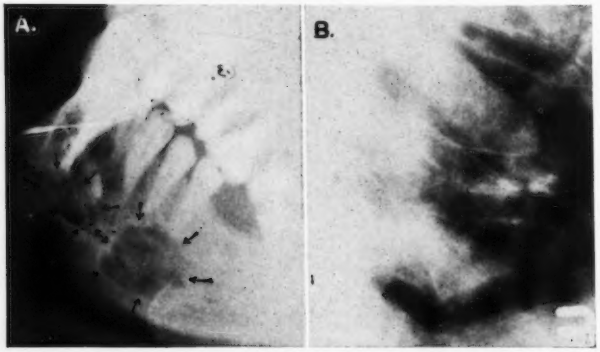

Fig. 4. *A.* Case 6. Roentgenogram of jaw. Note cysts.
B. Case 7. Roentgenogram of vertebrae showing two almost completely collapsed.

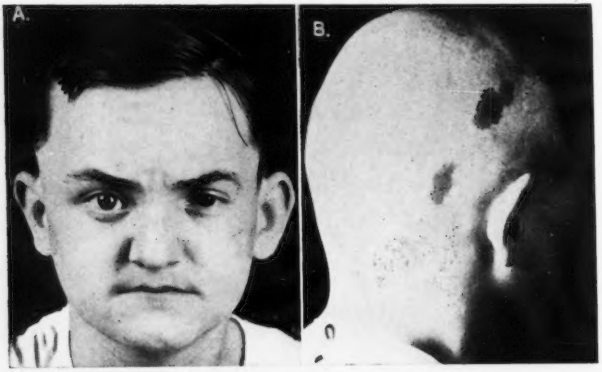

Fig. 5. *Case 7. A.* Note exophthalmos of right eye, suggesting possibility of Schüller-Christian disease on first examination. See discussion of possible relationship of syndrome to xanthomatosis in previous publication (1).
B. Photograph showing areas of pigmentation confined to scalp.

The fractures always healed rapidly. There was no family history of bone disease. The past history was irrelevant. The patient had always had areas of brown pigmentation. Puberty had developed about the normal time.

On physical examination, there was assymetry of the skull, marked deformity and shortening of the right leg, and areas of pigmentation confined mostly to the right side of the body, except for those on the scalp which were bilateral (fig. 2). The prostate was normal in size but the testes rather small.

The neurological examination brought out the very interesting fact that the right abdominal and cremasteric reflexes were absent, while those on the left were normal

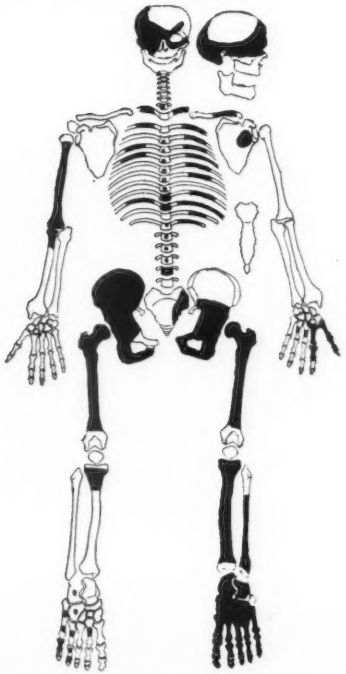

Fig. 6. Diagram showing distribution of skeletal lesions in *Case 7*.

Fig. 7. *Case 7*, A. Roentgenogram of pelvis. Note marked cystic degeneration of upper ends of both femora. Precocity of bone age is shown by the presence of the epiphysis of the crest of the ileum, which usually does not appear until about the age of 20.
B. Roentgenogram of skull. Note characteristic marked thickening of bones at base of skull.

(see distribution of pigment fig. 2). In addition there was diminished sensation to pin prick on the right, over the area supplied by the fourth dorsal to the first lumbar nerves.

In the laboratory studies, hematology and urine analyses gave normal findings; serum calcium was 10.7 mg. and inorganic phosphorus 2.7 mg. per 100 cc.; protein

was 7.0 mg. per 100 cc.; phosphatase 27 Bodansky units; nonprotein nitrogen 20 mg. per 100 cc.; chloride 105 m.eq. per liter; and total cholesterol 142 mg. per 100 cc. Gastric-analysis findings were normal; the glucose tolerance test showed normal or

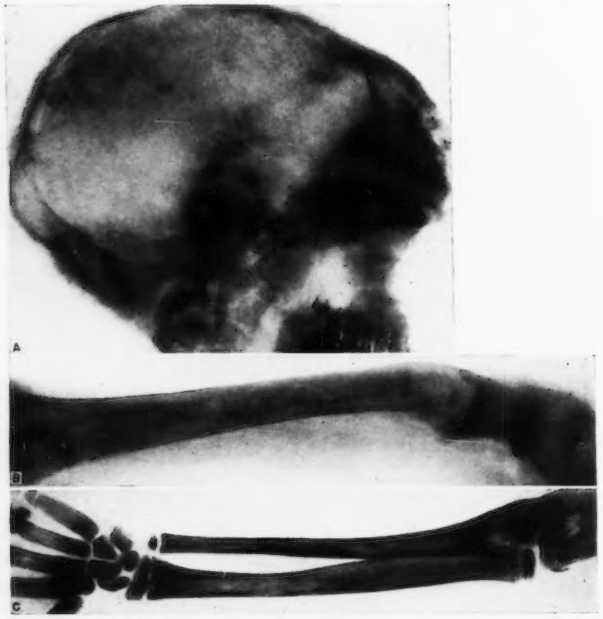

Fig. 8. Roentgenogram of *A*, skull, *B*, leg and *C*, arm bones of case of Musser and Barnwell.

Fig. 9. Showing pigmentation over buttock in Musser and Barnwell's case.

slightly decreased tolerance; the spinal fluid was normal; the B.M.R. was +8 per cent. No FSH was found in any of 3 first morning specimens of urine. The semen specimen was normal. The patient's I.Q. (Stanford-Binet) was 90. While on the ward he exhibited marked emotional instability.

The distribution of the bone lesions, as demonstrated by the roentgenograms is shown in figure 1. Individual lesions are shown in figures 3 *A, B,* and *C,* and 4*A.* Attention is called to the increase in thickness of both frontal bones. A biopsy of the skin through an area of pigmentation showed an increase of melanin in the basal layer.

The absence of hypercalcemia, the presence of areas of increased bone thickness and density, the tendency to a unilateral distribution of bone lesions, and the pigmented spots made it quite clear that this patient did not have hyperparathyroidism, but did have the syndrome described by Albright, Butler, Hampton, and Smith (1). The high serum phosphatase was consistent with both conditions. The only treatment therefore advised was an orthopedic operation to correct the deformity in the right femur.

At a follow-up examination in Sept. 1937, the same neurological abnormalities were elicited. The patient had developed slight bilateral gynecomastia, a finding for which no explanation was apparent.

Case 7. J. B. M. (M.G.H. No. 35885), a boy aged 14 years, entered the Massachusetts General Hospital at the request of Dr. Elias S. Faisen of Fort Mill, S.C., on April 2, 1937. The admission diagnosis was hyperparathyroidism.

The present illness started at the age of 2, when the patient developed bowed legs and a waddling type of gait. At 7, his right eye began to protrude, and ever since then it has remained prominent (fig. 5*A*). At 10, there was a spontaneous fracture of his right hip. Although this healed with the help of a cast, he has required crutches. At 12, on the basis of bone changes his parathyroid glands were explored, but no tumor was found. Following this, an operation was performed on each leg with a view to correcting the deformities. Puberty developed at 12.

The past history was irrelevant. There was no history of polyuria, polydipsia, or renal colic.

On physical examination, there was a marked prominence of the right side of the head (fig. 5*A*) with a dilated right pupil and a prominent right eye. Both pupils reacted to light and in accommodation. There were two areas of brown pigmentation in the right occipital region (fig. 5*B*), and deformities of both hips (fig. 7*A*). The testes and penis were normal for an adult man, and possibly over-developed for a boy of his age. The deep reflexes of his legs were markedly hyperactive, but the Babinski reflex was normal, and there was no clonus. Deep reflexes of his arms were likewise active, as were his abdominal and cremasteric reflexes.

Laboratory studies showed that, per 100 cc. of serum, there were 10.1 mg. of calcium, 3.2 mg. of inorganic phosphorus, 7.3 gm. of protein, 175 mg. of total cholesterol, the equivalent of 101 cc. of 0.1 N chloride, and 18 mg. of nonprotein nitrogen, all normal values. The serum phosphatase level was increased to 13.1 Bodansky units. Phenolsulphonephthalein was excreted normally by the kidneys. The Wassermann reaction was negative. The blood and urine findings were normal. By rough tests (2), there was no increase of calcium in the urine. The spinal fluid was normal.

The distribution of the skeletal changes as demonstrated by roentgenograms is shown in figure 6. The upper ends of both femora showed marked bowing (fig. 7*A*), due to numerous large and small cyst-like areas. The pelvis and left side of the sacrum were also markedly deformed. The lumbar and dorsal vertebrae had a very abnormal appearance, and there were two collapsed wedge-shaped vertebrae (fig. 4*B*). A definite abnormality showed in the region of the pedicle and lamina of the first thoracic vertebra on the left side, in that the pedicle was either destroyed or

markedly expanded and thinned. Similar less marked changes in the pedicles of several other thoracic vertebrae were present, and suggested the possibility of cord compression. The ribs showed irregular thickening, particularly on the right side and in the anterior ends of the first ribs. The bones of the left hand showed thickening and elongation of the first and second metacarpals, with obliteration of the medullary canal by homogeneous bone, and there were similar less marked changes in the phalanges of the first, second, and third fingers, whereas the bones of the right hand were normal in appearance. The skull showed marked thickening of the base and of the occiput and frontal bone, and other bones were likewise involved (fig. 7*B*). The bone age was about 3 years precocious. The teeth were not definitely abnormal.

A bone biopsy, taken from the skull by Dr. Oliver Cope, showed characteristic osteitis fibrosa, and no evidence of xanthomatosis.

The diagnosis of the syndrome under discussion seemed assured, and the patient was discharged on April 15, 1937, unimproved.

DISCUSSION

In the previous paper (1) based on 21 cases, inclusive of those found in the literature, it was emphasized that the syndrome in males did not cause sexual precocity. That series included 16 females, 12 of whom had definite sexual precocity, and 5 males, in none of whom precocity was observed. Furthermore, in the one male reported in that paper, the finding of small testes combined with normal secondary sexual characteristics was noted. It is therefore of interest that the semen specimen in *case 6*, here reported, was normal. It should also be noted that the bone age in *case 7* was definitely precocious, and the age of onset of puberty (12 years) was at the lower range of normal. There was no evidence of sexual precocity in *case 6*. It would therefore seem that this syndrome may cause slight precocity even in the male, although probably not to the same degree as in the female.

Probably the most important findings in the 2 cases here reported are the neurological changes in *case 6*. They furnish the first objective evidence of a neurological lesion roughly corresponding in distribution to the cutaneous and skeletal lesions, and help strengthen the hypothesis that some neurological lesion is at the basis of the whole syndrome (see above). The emotional instability of *case 6* should also be mentioned in this connection.[3]

It becomes of interest to pursue somewhat further the possibility that the precocity seen in this syndrome may be due to a central nervous system lesion rather than to a primary lesion of the endocrine system. Ford and Guild (3), who recently called attention to the fact that injuries involving the region of the hypothalamus may be followed by precocious puberty, reported 2 cases in which measles encephalitis was followed by precocious puberty in girls, and 1 case in which epidemic encephalitis was followed by precocious puberty in a boy. They made the interesting statement that not a "single case has yet been described in which precocious sexual development has been definitely connected with hypophyseal tumors of any type." They also cite evidence leading to the conclusion that the precocious puberty seen in cases of pineal tumors is due, neither to a hormone produced by that tumor, nor to

[3] A very similar mental picture has been encountered in another case studied subsequently.

the destruction of the pineal by the tumor, but to involvement of the walls of the third ventricle and hypothalamus by the tumor. Therefore, it seems conceivable that the endocrine disturbance in the syndrome under discussion may be the result of some brain lesion, presumably in the hypothalamic region. It is of great interest, in view of our series, that 2 of the cases cited by Ford and Guild were females, since most of the other cases in the literature with brain lesions and precocity were males. This is especially true with the 'pineal syndrome.'

None of the cases seen in this clinic have had hyperthyroidism. This was a prominent feature of McCune's case (4), however, and Barnwell's boy (5) died as the result of a thyroid operation. The occurrence of 2 instances of hyperthyroidism in such a small series (25 cases having come to the atten-

TABLE 1. METABOLIC DATA ON *case 6*

Period	Date	Calcium, gm. per day				Phosphorus, gm. per day				Serum, mg. per 100 cc.	
		Urine	Feces	Intake	Bal.	Urine	Feces	Intake	Bal.	Ca	P
I	March 1937										
	15–16–17	0.19	0.15	0.10	−0.24	0.50	0.25	0.59	−0.16	10.7	2.7
II	18–19–20	0.26	0.10	0.10	−0.26	0.42	0.19	0.59	−0.02		
III	21–22–23	0.24	0.12	0.10	−0.26	0.48	0.22	0.59	−0.11		
Normal values (6)		0.04	0.11	0.11	−0.04	0.40	0.20	0.69	0.09		

tion of the authors) brings up the possibility of a concomitant disturbance of the thyreotropic hormone of the anterior pituitary in some patients with this syndrome.

In table 1 are shown the metabolic data in *case 6*. It will be noted that the calcium excretions in the urine are much increased as compared with those of normal controls. This finding is at variance with similar studies on 3 of the patients reported in the first communication (1). The discrepancy can probably be explained, as the patient in *case 6* had much more bone disease than 2 of these 3 patients (*cases 1* and *2*). The third of the 3 patients (*case 5*) had extensive bone lesions, but these had been quiescent for a long time, and the roentgenograms showed a predominance of areas of increased bone density.

These metabolic findings are exactly what one would expect from the histology of the bones. They are important because they show that the presence or absence of hypercalcinuria will not serve to differentiate the condition from hyperparathyroidism.

SUMMARY

Two more case histories in males are recorded of a syndrome characterized by osteitis fibrosa disseminata, areas of cutaneous pigmentation, and marked sexual and somatic precocity when the disease occurs in females. In the case of one male reported, the bone age was precocious, suggesting that even in

the male sex some precocity may occur. In another case, on the same side as the cutaneous lesions and the majority of the bone lesions, abdominal and cremasteric reflexes were absent and there was decreased pain sensation. These findings strengthen the hypothesis that the whole syndrome is due to a disseminated neurological lesion.

Evidence from the literature is cited showing that sexual precocity may be due to lesions of the walls of the third ventricle and of the hypothalamus. Attention is called to the fact that 2 patients with the syndrome had hyperthyroidism as well. Metabolic studies on 1 patient showed that more than the normal amount of calcium was excreted in the urine.

APPENDIX

The discussion to this paper was opened by Dr. Roy Barnwell of Akron, Ohio, who showed some colored motion pictures of a patient undoubtedly belonging to the syndrome.[4]

The patient, a boy aged 11 years, entered the Children's Hospital in Akron as a patient of Dr. H. H. Musser because of a fracture of his left femur, which had followed an insignificant fall. Four years previously, he had also been treated at the same hospital for a fracture of the left femur, at which time his head was already enlarged. Since this former admission, he had developed an enlarged thyroid, and during the past 18 months had been under treatment for hyperthyroidism. Puberty had not yet occurred.

The points of interest on physical examination were a markedly enlarged head (fig. 8) with prominent posterior and frontal bossae; a deformity of the right hip (fig. 8, *B*); areas of brown pigmentation over both sides of face, neck and upper thorax, over the right shoulder and arm, and over the right buttock (fig. 9); a thyroid about twice normal in size, the right lobe being larger than the left; an enlarged heart with a rapid pulse (128); and a blood pressure of 154 systolic and 70 diastolic.

Roentgenograms showed widespread bone lesions of the type characteristic of this disease (fig. 8). The skull was of special interest because of the extreme thickness of the frontal bone (fig. 8). The serum calcium level varied between 8.7 and 12.2 mg. per 100 cc., and serum phosphorus level between 2.6 and 3.6 mg. per 100 cc. Two B.M.R. values were plus 40 per cent and plus 60 per cent, and the blood cholesterol was 78 mg. per 100 cc.

A diagnosis of hyperparathyroidism was entertained, and a parathyroid operation was being considered, until the paper by Albright, Butler, Hampton and Smith (1) appeared, at which juncture the patient was recognized as belonging to the syndrome under discussion.

Subsequent to the Atlantic City Meeting, the severe hyperthyroidism in this patient required an operation, which he did not survive, dying 3 hours postoperatively. One normal parathyroid gland was encountered at the operation, but no parathyroid adenomata. On histological examination the thyroid was not characteristic of that seen in Graves's disease, but suggested a diffuse colloid goiter.

Unfortunately, autopsy did not include the brain, spinal cord, or pituitary. The adrenal glands showed no marked abnormality, and the testes were normal in size for his age. The thymus was considered unusually large. Three small additional

[4] The authors wish to express their appreciation to Dr. H. H. Musser and to Dr. Roy Barnwell for permission to include in this report these notes concerning their most interesting patient.

masses of parathyroid tissue were found after prolonged search, but nothing to suggest a parathyroid adenoma. There was dilatation of the right side of the heart, and chronic passive congestion of multiple organs.

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CLINICAL RESULTS OF ANTERIOR PITUITARY THERAPY IN CHILDREN

A COMPARISON OF THE VALUE OF ORAL AND HYPODERMIC PREPARATIONS

MATTHEW MOLITCH AND SAMUEL POLIAKOFF
From the Department of Neurology, School of Medicine, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA
and the New Jersey State Home for Boys
JAMESBURG, NEW JERSEY

Many clinicians during the past few years have reported their results from the administration of anterior pituitary growth fractions in children or young adults below the optimum height for their age. These reports covering 1 to 10 or more patients, include a group of 5 successfully treated by the authors (1). The most recent report by Jacobson and Cramer (2) on a variety of conditions does not lend itself to comparison with previous publications. Shelton, Cavanaugh and Evans (3) recently published a critical report on the extensive use of anterior pituitary growth fractions on 6 patients over a period of nearly 5 years. They concluded that the extracts employed by them will promote growth when administered intramuscularly; that, from a purely clinical standpoint, the growth-promoting property is disappointing; that the preparations are not sufficiently active to rehabilitate the classic dwarf, but may be clinically useful in the rehabilitation, during the growing period, of dwarfish persons not losing over 50 per cent of the normal growth increment. They also felt that prolonged administration produces little evidence of a subsequent refractory state, and does not interfere with either the onset of puberty, or the spurt of growth which may occur in this period; but that prolonged and intensive administration is not without an element of physiologic danger.

The senior author has had opportunities, through correspondence and by word of mouth, to discuss the treatment of short children with many able clinicians from different parts of the country who are interested in endocrinology. One prominent observer has discontinued the use of so-called growth hormones. Several others report successful results, with the use of anterior pituitary substance by mouth, in the form of tablets. This diversity of opinion prompted the authors to try various methods of treatment, designed to stimulate growth, on several groups of children in a controlled environment. A similar group without treatment was used as a control.

Forty-three white boys between 10 and 17 years, all of whom were below the minimum in height for their age according to Englebach's (4) norms, were

selected for observation. Table 1 presents anthropometric data demonstrating that almost average normal boys were included in the experiment. The bone age of each boy was within limits, ruling out the probability of long-standing hypothyroidism (5). The standards used for bone age were those of Shelton (6).

TABLE 1. ANTHROPOMETRIC DATA BEFORE TREATMENT WAS STARTED

Case	Age	Height (inches)	Wt. (lb.)	Previous Annual Increment	Normal for Age (Englebach)					Inches Below Min. in Ht. for Age
					Height		Weight		Annual Optimal Increment	
					Min.	Max.	Min.	Max.		
				Controls						
T.V.	11-2	53½	69	1¼	53.3	57.1	63.0	79.4	2.0	0.1
D.S.	12-7	55¾	75	1¾	56.0	60.0	72.7	91.3	1.8	0.2
J.C.	14-1	58	78	1¾	58.6	62.8	81.1	102.9	1.8	0.6
J.M.	12-3	54¾	72	1¼	55.2	59.0	69.4	87.2	1.8	0.5
J.D.	13-3	55½	77	2¼	56.9	60.9	75.9	95.7	1.8	1.4
J.Y.	15-7	59¾	96	1¾	61.1	65.3	91.6	116.2	1.6	1.4
J.C.	15-6	58½	92	1½	61.1	65.3	91.6	116.2	1.6	2.6
M.C.	12-4	52½	75	1	56.0	60.0	72.7	91.3	1.8	3.5
A.S.	12-6	53	67	1	56.0	60.0	72.7	91.3	1.8	3.0
C.L.	12-10	54¼	71	1½	56.9	60.9	75.9	95.7	1.8	2.7
L.P.	15-9	54¼	73	2	61.8	66.2	96.0	122.0	1.6	7.6
				Thyroid Treatment Only						
L.P.	14-2	58¼	90	1¾	58.6	62.8	81.1	102.9	1.8	0.4
S.H.	15	59½	93	1½	60.3	64.5	89.4	113.4	1.6	0.8
A.N.	13-2	56	87	1½	56.9	60.9	75.9	95.7	1.8	0.9
D.D.	14-8	58	83	2	59.5	63.7	89.4	113.4	1.7	1.5
E.C.	14	57	84	1½	58.6	62.8	81.1	102.9	1.8	1.6
R.C.	15-1	58¼	81	¾	60.3	64.5	89.4	113.4	1.6	2.1
E.M.	15-2	57¾	81	1¼	60.3	64.5	89.4	113.4	1.8	2.6
W.C.	14-3	55¼	70	½	58.6	62.8	81.1	102.9	1.8	3.2
C.G.	12-4	52¾	64	1½	56.0	60.0	69.4	87.2	1.8	3.3
A.S.	14-0	54½	70	1½	58.6	62.8	81.1	102.9	1.8	4.1
J.D.	15	56¼	100	1½	61.8	66.2	96.0	122.0	1.6	5.6
				Thyroid and Pituitary Tablets						
J.T.	12-7	55¾	90	2	56.0	60.0	72.7	91.3	1.8	0.2
C.M.	15-10	61	98	2¼	61.8	66.2	96.0	122.0	1.6	0.8
J.C.	13-6	56¾	85	1	57.8	61.8	79.3	100.3	1.8	1.1
E.P.	14-4	58	82	1¾	59.5	63.7	85.0	108.0	1.8	1.5
W.J.	14-10	58¾	90	1½	60.3	64.5	89.4	113.4	1.7	1.6
S.L.	12-5	53¾	76	1½	56.0	60.0	72.7	91.3	1.8	2.3
J.V.	9-11	49	66	¾	51.4	55.0	57.0	71.6	2.0	2.4
R.W.	15	57¼	80	2	60.3	64.5	89.4	113.4	1.6	3.1
E.J.	13-3	53¾	65	1½	56.9	60.9	75.9	95.7	1.8	3.2
G.M.	17-2	58	86	1	63.2	67.6	103.8	131.6	1.3	5.2
F.DeR.	13-6	52	77	1¼	57.8	61.8	79.3	100.3	1.8	5.8
				Thyroid and Pituitary Extract						
J.C.	12-2	55	82	2	55.2	59.0	69.4	87.2	1.8	0.2
W.H.	15-8	60	93	—	61.1	65.3	91.6	116.2	1.6	1.1
J.D.	13-11	57	83	1½	58.6	62.8	81.1	102.9	1.8	1.6
W.P.	14-6	57¾	98	4(?)	59.5	63.7	85.0	108.0	1.7	1.8
G.L.	14-5	57½	90	1	59.5	63.7	85.0	108.0	1.7	1.8
A.K.	15-8	58	84	1½	61.1	65.3	91.6	116.2	1.6	3.1
S.C.	15-10	58¼	101	3(?)	61.8	66.2	96.0	122.0	1.5	3.6
M.R.	13-8	54½	68	1¼	57.8	61.8	79.3	100.3	1.8	3.6
A.S.	14	52½	67	2	58.6	62.8	81.1	102.9	1.8	6.4
D.M.	14-10	53¾	70	—	60.3	64.5	89.4	113.4	1.7	6.6

No known cause for their short stature was discovered, and it was concluded that they were probably suffering from some degree of hypophyseal infantilism. The deficiencies ranged from 0.1 to 7.6 inches below the minimum in height for their age, giving us an opportunity to determine the response in children with varying degrees of growth deficiency. Table 2 shows the distribution of the boys into 4 groups. Eleven boys in the first group received no treatment at all, and were used as controls. The second group of 11 boys received one gr. of thyroid gland substance daily without other medication, for a period of 6 months. A third group of 10 boys received 15 gr. of anterior pituitary lobe substance,[1] said to be equal to 75 gr. of the dried gland daily, in addition to one gr. of thyroid gland substance. The fourth and last group was given an active APE intramuscularly in doses 3 cc. (30 R.U.) 3 times weekly, together with one gr. of thyroid gland substance daily. The thyroid

TABLE 2. RÉSUMÉ OF ANTHROPOMETRIC DATA BEFORE TREATMENT

Groups	Total No. of Cases	Inches Below Minimum in Height for Age						
		0–1	1–2	2–3	3–4	4–5	5–6	6–7
Control	11	4	3	1	2	—	1	—
Thyroid	11	3	2	2	2	1	1	—
Thyroid and Pituitary Tablets	10	1	3	2	2	—	1	1
Thyroid and Pituitary Extract	11	2	3	2	2	—	2	—
Totals	43	10	11	7	8	1	5	1

TABLE 3. CONTROL CASES WHO RECEIVED NO TREATMENT

Case	Gain in Ht. during last 6 months	Case	Gain in Ht. during last 6 months	Case	Gain in Ht. during last 6 months
T.V.	¾	*A.S.*	½	*J.C.*	1
J.M.	½	*D.S.*	¾	*J.C.*	¾
M.C.	½	*C.L.*	¾	*J.Y.*	1¼
		J.D.	1	*L.P.*	¾

TABLE 4. CASES TREATED WITH THYROID GLAND SUBSTANCE ALONE FOR 6 MONTHS

Case	Thyroid gr.	Results after Treatment			
		Ht. in.	Wt. lb.	Gain in Ht. in.	Change in Wt. lb.
C.G.	177	53¾	63	1	−1
A.N.	176	56¾	85	¾	−2
E.C.	172	58½	88	1½	+4
A.S.	173	55¼	73	¾	+3
L.P.	175	59¾	93	1½	+3
W.C.	177	55½	70	¼	0
D.D.	167	59½	90	1½	+7
S.H.	167	61¼	100	1¾	+7
R.C.	177	58½	78	¼	−3
E.M.	172	59	81	1¼	0
J.D.	170	58	104	1¾	+4

[1] The thyroid and anterior pituitary lobe gland substances were supplied by the courtesy of Messrs. Zeller and Bradshaw of Armour Laboratories. The growth hormone was supplied by the courtesy of Dr. Anderson and Dr. Morrell of E. R. Squibb & Sons.

gland substance was given in conjunction with the pituitary medication, which is accepted by many as enhancing the value of these preparations.

Tables 3, 4, 5, and 6 give the details of the cases in the 4 groups under observation for 6 months. Table 4 shows that the boys in this group received an average of 173 gr. of thyroid gland substance. Table 5 shows that the boys

TABLE 5. CASES TREATED WITH THYROID SUBSTANCE ORALLY AND ANTERIOR PITUITARY EXTRACT TABLETS FOR 6 MONTHS

Case	Thyroid gr.	Pituitary Substance gr.	Results after Treatment			
			Ht. in.	Wt. lb.	Gain in Ht. in.	Change in Wt., lb.
J.V.	177	2655	50¼	65	1¼	−1
S.L.	174	2610	54¾	74	1	−2
J.T.	173	2585	56¾	86	1	−4
E.J.	174	2605	54½	66	¾	+1
F.D.	177	2655	52¾	77	¾	0
J.C.	177	2655	57¾	84	1	−1
E.P.	177	2655	60¼	87	2¼	+5
W.J.	177	2655	60½	88	1¾	−2
R.W.	175	2615	58¾	83	1½	+3
C.M.	175	2625	62¾	106	1¾	+8
G.M.	170	2550	59½	90	1½	+4

in the second group received an average of 175 gr. of thyroid gland substance and an average of 2624 gr. of anterior pituitary lobe substance. Table 6 shows that the boys in the last group received an average of 170 gr. of thyroid gland substance and 1769 R.U. of growth hormone. Table 7 gives a comparison of the results in all groups. It may be seen that those receiving medication apparently grew slightly more than the control group. In individual cases,

TABLE 6. CASES TREATED WITH THYROID SUBSTANCE AND ANTERIOR PITUITARY LOBE EXTRACT HYPODERMICALLY FOR 6 MONTHS

Case	Thyroid gr.	R.U.	Results after Treatment			
			Ht. in.	Wt. lb.	Gain in Ht. in.	Change in Wt., lb.
J.C.	177	1900	56	85	1	+3
M.R.	171	1900	55¼	66	1	−2
J.D.	143	650	58	88	1	+5
A.S.	174	1910	54½	71	2	+4
G.L.	168	1900	58½	89	1¾	−1
W.P.	176	1870	60¼	106	2½	+8
D.M.	168	1910	55	68	1¼	−2
A.K.	177	1900	58	84	¼	+3
W.H.	172	1850	61	96	1	+3
S.C.	177	1900	60¼	108	2	+7

however, it would appear that the greatest response followed the use of pituitary substances in addition to the thyroid substance. Table 8 gives an idea of the response of the children according to the degree of their shortness. Our findings are in accordance with those of others, namely, that the nearer a child is to normal the better will be his response to treatment. In addition, 6 of the boys in the 3 treated groups showed an increase in pubic hair and

genital development. One of the boys, whose scalp hair was sparse, developed a luxurious growth of hair during the course of treatment (fig. 4).

We feel that the hypodermic administrations of the anterior pituitary were more effective than the oral administrations in the entire group, especially in

Fig. 1. *Case J. D.* Treated with thyroid alone.
Fig. 2. *Case W. P.* Treated with anterior pituitary extract and thyroid.
Fig. 3. *Case S. C.* Treated with anterior pituitary extract and thyroid.
Fig. 4. *Case W. H.* Treated with anterior pituitary extract and thyroid.

selected cases. From this study it would appear that the oral preparation is of value and suggests the use of the hypodermic preparation only when medication by mouth has failed to stimulate growth. There were no unfavorable local and systemic reactions to treatment. The weight of the patient was influenced only slightly.

SUMMARY

Thirty-two boys, below the minimum in height for their age, were divided into 3 groups and treated for 6 months. Group *A* received thyroid alone, group *B* received thyroid plus anterior pituitary substance by mouth, group *C* received thyroid plus an A.P.E. intramuscularly. Eleven additional boys were not treated, but served as a control group.

The 'treated' children had a greater increment in height than the control

TABLE 7. COMPARISON OF RESULTS OF 6 MONTHS OBSERVATION AND TREATMENT

Group	Gain in Height (inches)										Median Response (inches)
	¼″	½″	¾″	1″	1¼″	1½″	1¾″	2″	2¼″	2½″	
Control	—	4	4	2	1	—	—	—	—	—	¾
Thyroid alone	2	—	2	1	1	3	2	—	—	—	1¼
Thyroid and Pituitary Tablets	—	—	2	3	1	2	2	—	1	—	1¼
Thyroid and Pituitary Extract	1	—	—	4	1	—	1	2	—	1	1⅜

TABLE 8. COMPARISON OF DEGREE OF DEFECT AND DEGREE OF RESPONSE TO TREATMENT

Inches Below Minimum		¼″	½″	¾″	1″	1¼″	1½″	1¾″	2″	2¼″	2½″
Control Group	0–1	—	—	2	1	1	—	—	—	—	—
	1–2	—	1	1	1	—	—	—	—	—	—
	2–3	—	—	1	—	—	—	—	—	—	—
	3–4	—	2	—	—	—	—	—	—	—	—
	5–6	—	1	—	—	—	—	—	—	—	—
Thyroid Alone	0–1	—	—	1	—	—	1	1	—	—	—
	1–2	—	—	—	—	—	2	—	—	—	—
	2–3	1	—	—	—	1	—	—	—	—	—
	3–4	1	—	—	1	—	—	—	—	—	—
	4–5	—	—	1	—	—	—	—	—	—	—
	5–6	—	—	—	—	—	—	1	—	—	—
Thyroid and Pituitary Extract	0–1	—	—	—	1	—	—	—	—	—	—
	1–2	—	—	—	2	—	—	—	—	—	1
	2–3	1	—	—	—	—	—	1	—	—	—
	3–4	—	—	—	1	—	—	—	1	—	—
	5–6	—	—	—	—	1	—	—	1	—	—
Thyroid and Pituitary Tablets	0–1	—	—	—	1	—	—	1	—	—	—
	1–2	—	—	—	1	—	—	1	—	1	—
	2–3	—	—	—	1	1	—	—	—	—	—
	3–4	—	—	1	—	—	1	—	—	—	—
	5–6	—	—	1	—	—	1	—	—	—	—

cases. Children whose growth was most rapid appeared to get the most stimulation. The outcome on all treated cases is so close that the results may be within the range of error. It would have been more satisfactory if the experiment had been carried out over a longer period of time, but this was impractical.

The thyroid gland substance may have stimulated growth, even though the patients did not have a retarded bone age. Anterior pituitary substances, when given concomitantly with thyroid by mouth and by hypodermic, produced better results than thyroid gland substance alone. Anterior pituitary sub-

stances given orally appeared slightly less effective than the extract given intramuscularly. The hypodermic preparation is recommended for use when the oral preparation has failed to stimulate growth.

I wish to thank Dr. E. Kost Shelton for his valuable advice and criticism.

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PROLACTIN—ITS EFFECT ON THE SECRETION OF WOMAN'S MILK[1]

JOHN R. ROSS

From the Laboratories of the Department of Paediatrics, University of Toronto, and Hospital for Sick Children

TORONTO, CANADA

From the dawn of medical history, an intensive effort has been put forward to find a treatment which would increase the supply of breast milk. Substances recommended for this purpose have varied from powder-of-earthworms in a soup made of cow's tongue to powdered quartz suspended in wine. Even in recent years many proprietary substances have been advertised for this purpose, but there has been little or no scientific basis for their use. Any beneficial effects obtained were probably due to the psychic factor.

In 1928, Stricker and Gruter (1) reported that extracts of the anterior lobes of beef pituitary glands injected into suitably prepared rabbits will cause lactation. Since that time, lactation has been initiated in other species (rat, guinea pig, goat, bitch, sow) by workers like Corner (2), Evans and Simpson (3, 4), Nelson and Pfiffner (5), Turner and Gardner (6,7), Putnam, Benedict and Teel (8), Gruter (9) and Asdell (10), using crude extracts of beef or sheep pituitaries.

Riddle, Bates and Dykshorn (11, 12) presented strong evidence that the pituitary substance responsible for the initiation of milk secretion was neither growth, gonad-stimulating, luteinizing, nor thyrotropic hormone, but a hitherto unseparated anterior lobe hormone to which they gave the name prolactin. This same substance was shown to cause the production of 'crop milk' in doves and pigeons (an analogous response to the secretion of milk by mammals) and they also outlined a method of assay, which has since been widely adopted, based upon the ability of this hormone to stimulate growth and proliferation of the crop-glands of immature male and female doves and pigeons. They define their unit as that amount of hormone which is equivalent in crop-gland stimulating activity to 1 mg. of their preparation No. 51, and this is likewise the minimum quantity producing a detectable crop-gland response.

In January 1934, Kurzrok, Bates, Riddle and Miller (13) published a preliminary report on the use of prolactin in stimulating the breast milk secretion of nursing mothers. They chose 29 cases in which lactation had failed to develop by the sixth day or later post partum, and which in the clinical experience of the staff would in almost every instance have failed further to improve in milk production. They found that in 21 of 29 such cases the breast milk output was increased over 100 gm. daily by the injection of 75 to 400 U. of prolactin, and in only 4 cases was there complete failure to increase the daily output of breast milk. The amount of milk

[1] Read at the Fifteenth Annual Meeting of the Canadian Society for the Study of Diseases of Children, Niagara Falls, Ont., June 11 and 12, 1937.

secreted was judged by the difference in weight of the baby before and after nursing. Their results were classified as follows. An increase of 0 to 50 cc. in the amount of breast milk, secreted on the last day before discharge as compared to the day before the injection was given, was classified as a failure; increases of from 50 to 100 cc. were considered moderately successful, and gains over 100 cc. were classed as good results.

In order to obtain further information on the efficacy of this galactagog, the present author has made observations on nursing mothers at the Burn-

TABLE 1

Case Number	Dosage		Gm. Milk in Day		Days after Prolactin (Date of Discharge)
	Prolactin Units	On Day Postpartum	Day before Prolactin	Day of Discharge	
1	400	6	148	137	6
2	400	8	126	464	4
3	400	7	330	594	6
4	400	8	236	609	4
5	400	10	238	212	2
6	400	6	125	126	6
7	400	8	345	418	6
8	400	6	210	246	3
9	400	6	386	874	4
	Average		235	409	
10	1000	7	238	404	7
11	1000	7	380	738	5
12	1000	7	290	433	7
13	1000	8	235	396	6
14	1000	7	232	468	2
15	1000	7	312	472	7
16	1000	8	264	397	5
17	1000	8	347	450	4
18	1000	8	281	604	6
19	1000	7	128	472	4
20	1000	9	248	464	5
21	1000	8	104	265	4
	Average		255	464	
22	0	6	90	324	7
23	0	7	372	510	8
24	0	6	108	191	7
25	0	9	214	210	2
26	0	7	200	297	6
27	0	7	337	518	5
28	0	7	168	438	5
29	0	9	490	589	4
30	0	8	326	520	6
31	0	7	272	120	6
	Average		248	376	

side Obstetrical Department of the Toronto General Hospital.[2] The breast milk was pumped by an Abt electric breast pump, and the amount obtained carefully measured. By the use of the breast pump it was possible to standard-

[2] I am indebted to Professor W. A. Scott, Obstetrician-in-Chief, for permission to conduct these observations, and to Miss Kelly, nurse-in-charge of the hospital, for her assistance and coöperation.
The urine assays were done in the Research Laboratory of Ayerst, McKenna and Harrison, Ltd., through the courtesy of Dr. A. Stanley Cook.

ize the intensity and duration of the suction used in emptying the breast. As the amount of breast milk secreted is dependent to a measure on the stimulus received, it would seem important to standardize this stimulus. During the period of observation, a maximum of 4 babies was observed at any one time. The mothers and infants under observation were cared for by 3 nurses who made this work their sole duty. As the nurses worked on 8-hour shifts, there was one nurse at all times carrying out the observations. Apart from this, control and experimental groups were kept on the customary hospital routine as regards diet and management.

Infants were selected who did not show signs of weight increase by the fifth day. Breast milk was then pumped from the mother, and the total 24-hour secretion noted for 1 or usually 2 days. Mothers whose infants are gaining normally secrete on the average 550 cc. on the sixth day. If the selected mothers secreted less than 400 cc. on the sixth day, they were then put into 1 of 3 groups.

The first group received on the first day 2 intramuscular injections of 2 cc., and on the second day 2 intramuscular injections of 3 cc., making a total over a 2-day period of 10 cc. of prolactin[8] containing 40 Riddle U per cc. The second group received a similar volume of prolactin containing 100 Riddle U. per cc., or a total of 1000 U. over a 2-day period. In most of the cases in this group, the two 3-cc. injections were given on the first day, and the two 2-cc. injections on the second day. The third group acted as controls, and received a similar amount of saline. In order to remove any psychic influence, the saline was sealed in ampules and labelled prolactin-A (prolactin absent), so that even the nurses administering the material had no idea that in these cases no prolactin was being administered.

The breast milk was pumped at 4-hourly intervals until the patient was discharged. The number of controls were 10; 9 patients received the smaller dose of prolactin, and 12 the larger dose. No untoward reactions were noted with the less concentrated prolactin. With the more concentrated prolactin, however, reactions consisting of redness and induration varying in diameter from 2 to 15 cm. developed in 83 per cent of the cases. These cleared up in 2 to 3 days. There was also a rise in temperature to 100° or 102°F. in all but one case following the injections, but a return to normalcy occurred in 2 to 6 days.

RESULTS

The results obtained are tabulated in table 1. In table 2 the results are classified according to the standards used by Kurzrok, Bates, Riddle and Miller. It is evident from the results given in these tables that the injection of 400 U. of prolactin produced very little effect when compared with the control series. However, the larger amount of prolactin (1000 U.) appeared to result in an appreciable increase in breast milk secretion.

Of much greater importance, however, are the results of a survey of the mother's ability in the 3 different groups to nurse their infants after dis-

[8] The prolactin used in this investigation was prepared by the method of Bates and Riddle (14) and manufactured by Messrs. Ayerst, McKenna and Harrison, Ltd., Montreal.

charge from the hospital. As many mothers as possible were checked, at periods ranging from 3 weeks to 5 months after discharge, to determine their ability to nurse their infants. The results are given in table 3. It is evident that those mothers receiving the lower concentration of prolactin were not able to nurse their babies any better than those who had received injections of normal saline. In contrast to this, the results obtained with the mothers who received the higher concentration of prolactin are quite striking.

TABLE 2. INCREASE IN DAILY SECRETION OF BREAST MILK AFTER INJECTION OF PROLACTIN (DAY OF DISCHARGE FROM HOSPITAL)

	0–50 cc. Failure	50–100 cc. Moderate result	Over 100 cc. Good result
Kurzrok Cases:			
Prolactin	4	4	21
Our Cases:			
Prolactin 400 u.	3	2	4
Prolactin 1000 u.	0	0	12
Control	3	1	6

Table 4 gives the distribution of the patients according to their ages and number of pregnancies, showing that there was no great difference in the number of pregnancies and the ages of the mothers in the 3 different groups.

The fat, protein and ash content of the breast milk from practically all patients was determined. The average results given in table 5 show that the extra secretion obtained as the result of prolactin administration had no effect on the composition of the breast milk.

In view of the close inter-relationship of the anterior pituitary gland with the gonads, and the knowledge that estrin will inhibit lactation in animals,

TABLE 3. TYPE OF FEEDING USED 3 TO 20 WEEKS AFTER DISCHARGE FROM HOSPITAL

	Breast only	Breast and Supplement	Bottle only	Unable locate
Prolactin 400 u. (9 cases)	2	0	6	1
Prolactin 1000 u. (12 cases)	7	2	2	1
Control	1	3	4	2

it was thought that a study of the hormone excretion of treated and untreated patients who were lactating subnormally, as compared with normally lactating women, might give some clue as to the reason for the failure of lactation in some of the cases.

Twenty-four-hour urine specimens were collected on the fifth day postpartum (before treatment) and on the 10th or 11th day postpartum (after treatment). Since tests on the early cases indicated that the estrin content was low, the urine was extracted with butyl alcohol, the extract evaporated to dryness and the residue taken up in a minimal quantity of 10 per cent aqueous alcohol.

A quantity of the extract equivalent to 100 cc. of original urine was injected subcutaneously into 21-day-old female rats, the total dose being divided

into 6 injections, twice daily for 3 days. The volume of each injection varied from 0.2 to 0.4 cc. Vaginal smears were examined once on the fourth day, twice on the fifth, and once on the sixth after the first injection. Those samples which gave a positive response at a dose equivalent to 100 cc. original urine were tested at smaller doses, until a dose was reached which failed to give a positive response.

Six normally lactating women excreted less than 13 U. of estrin per day on the 5th and 11th days postpartum. Of the women who were not lactating normally, 3 excreted 28, 20 and 16 U. respectively on the fifth day postpartum,

TABLE 4. AGE AND PAROUS STATE OF MOTHERS UNDER OBSERVATION

	Number of Pregnancies		Age	
	Multipara	Primipara	15–30	Over 30
Prolactin 400 U.	5	4	7	2
Prolactin 1000 U.	7	5	8	4
Control	4	6	8	2

the remaining 16 excreting less than these amounts. On the 11th day, the highest excretion was 9 U. The patient who excreted 28 U. of estrin on the fifth day also excreted a trace (over 70 U.) of gonadotropic hormone on that day. On the 11th day this patient's estrin excretion had dropped to less than 10 U., and gonadotropic hormone was absent.

These results confirm those of other workers in showing how rapidly estrin excretion drops following parturition. The excretion of estrogenic material in these cases is of the same order as that encountered during the normal menstrual cycle.

TABLE 5. CHEMICAL COMPOSITION OF BREAST MILK

	Fat %		Protein %		Ash %	
	5th day	12th day	5th day	12th day	5th day	12th day
Patients given Prolactin 400 U.	2.3	3.0	2.0	1.6	.34	.28
Patients given Prolactin 1000 U.	2.7	3.3	1.7	1.3	.36	.26
Control patients	2.7	3.2	2.1	1.8	.38	.30
Normal patients	2.5	3.0	1.5	1.5	.31	.24

The reactions to the larger doses of prolactin encountered during this investigation were severe enough to limit seriously its use in general clinical practice. Further investigation should be made with a view to determining whether a different method of administration can be found which will decrease the occurrence of reactions while retaining the maximum effect of the hormone.

Bates and Riddle (15) have shown that injection by the subcutaneous route is 5 times more efficient than the intramuscular route in eliciting the crop-gland response in pigeons. They have also shown that dividing the daily dosage into 3 injections greatly increases that response. The maximum crop-

gland response is reached after injection for a period of 7 days. There is at present no information available as to the optimum route, frequency or duration of injections in humans. It is possible that equally good or better responses in women might be obtained by using smaller doses given subcutaneously twice or 3 times daily for a longer period, obviating in this way the reactions associated with larger doses of prolactin.

In general clinical practice, it should not be necessary to delay the commencement of treatment in all cases until the sixth day postpartum. Treatment could be commenced as soon as clinical experience indicated that the patient will probably not have sufficient milk.

SUMMARY

Prolactin was administered, intramuscularly to mothers whose daily breast milk secretion on the fifth and sixth days was less than 400 cc. The injections were given twice daily over a 2-day period. One group of patients received a total of 400 U., whereas the second group received a total of 1000 U. A control group of patients received intramuscular injections of normal saline.

The higher concentration of prolactin administered appeared to increase slightly the secretion of breast milk during the remainder of the stay in hospital. Of much more significance, however, was the fact that 7 out of 11 patients receiving this higher concentration of prolactin, whose records could be followed after discharge from the hospital, nursed their infants completely, whereas out of 8 such observed patients receiving the lower concentration of prolactin only 2 were able to nurse their infants completely. Of the 8 controls observed who received normal saline, only 1 nursed her infant completely.

Rather severe local reactions followed the injection of the larger amount of prolactin. It is probable that further purification of prolactin will eliminate these reactions. The administration of prolactin apparently has no effect on the fat, protein or ash content of the breast milk secreted. The galactagog effect obtained justifies the further investigation of the value of this product. The urinary excretion of estrin in these cases was within the range found during the normal menstrual cycle.

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THE BIOLOGICAL EFFECTS OF THYMUS IMPLANTATION IN THYMECTOMIZED RATS[1]

CORRECTION OF RETARDATION IN GROWTH AND DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMECTOMIZED RATS BY MEANS OF FREQUENT HOMOLOGOUS THYMUS IMPLANTS[2]

NATHAN H. EINHORN

From the Samuel Bell, Jr. Laboratory of the Philadelphia Institute for Medical Research in the Philadelphia General Hospital

PHILADELPHIA, PENNSYLVANIA

The success attending the use of thymus extract as substitution therapy in the treatment of thymectomized rats suggested the use of homologous thymus implants for the same purpose. Such an experiment would also throw light on the question whether the effects following the injection of thymus extract were due to a substance normally inherent in the thymus gland, or to some agent or principle produced during the process of extracting the thymus gland.

METHOD OF EXPERIMENTAL PROCEDURE

Accordingly, 5 pairs of albino rats (Wistar strain) of known parentage were selected from the normal stock animals of the Philadelphia Institute for Medical Research. Three pairs were thymectomized at the age of 21 days by the method described in an earlier paper, mated, and at 30 days started on weekly subcutaneous implants of 1 whole thymus gland obtained from stock rats 20 to 30 days of age (1). The remaining 2 pairs were subjected to the operative technique concerned in thymectomy, except that the thymi were not removed. These latter animals were designated operative controls. Data on somatic development were recorded daily, and the weights of the rats twice weekly, up to the age of 70 days. All the animals in one litter, or part of a large litter of each pair of parent animals, were thymectomized, mated, and set up as parents of the next generation. Thus, the offspring in each generation were young born to thymectomized parents receiving frequent homologous implants of whole thymi. Implantations were always started at the age of 30 days, and a total of 12 implants was given to each animal. Rats were housed and fed under the conditions previously described.

All of the implants were placed in the areolar tissue between the skin and muscles of the back. A definite plan was followed in placing each implant so that in every animal, the first implant was always over the right ileum, the

[1] This work was supported by a grant from the Penrose Fund of the American Philosophical Society and from the Rockefeller Foundation.

[2] Part of thesis studies submitted to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Doctor of Science, D.Sc. (Med.).

fourth on the right side of the neck, the eighth over the left ileum, etc. In figure 1 is shown the location of each of these implants.

The technique of implantation insured great speed in the transfer of the thymus gland from the donor to the recipient. The recipient was fastened securely on a specially constructed frame, the proper area on the back cleaned of hair, and infiltrated with 1 per cent procaine solution. A small V-shaped incision was made in the skin with scissors, and a subcutaneous pocket prepared by blunt dissection. A mattress suture was then placed in the edges of the incision, and the first knot tied. After preparation of the recipient was completed, the donor was stunned, its thymus removed in toto, transferred into

TABLE 1. SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMUS-IMPLANT-THYMECTOMY GROUP

		Ears opened	Upper incisors erupted	Hair appeared	Eyes open	Testes descended	Vagina opened
Operative Controls	Range	2–3	7–10	11–14	13–17	24–35	39–50
	Average	2.9	8.5	12.5	15.2	30.2	44.3
F_1	Range	3	7–9	12–14	12–16	25–31	36–46
	Average	3	8.1	13.2	15.1	28	42.2
	Average, Thymectomy Group[1]	3.4	9.4	14.8	15.9	30.4	45.1
F_2	Range	1–3	3–9	8–13	7–14	20–28	33–42
	Average	2.3	6	10.3	10.9	22.2	38.5
	Average, Thymectomy Group	3.3	9.5	14.2	16.2	30.7	52.0
F_3	Range	1–3	4–7	9–13	7–15	18–26	32–40
	Average	2	5.4	10.4	9.1	20.3	36.3
	Average, Thymectomy Group	3.2	9.6	13.6	16.3	30.4	46.5
F_4	Range	1–3	2–7	6–12	3–13	16–22	32–38
	Average	1.9	4.5	9	7.6	19.2	34.2
	Average, Thymectomy Group	3.5	9.6	14.8	16.8	30.0	48.8

All figures represent days.
[1] Untreated, and without implants.

the previously prepared pocket, and the ligature in the skin of the recipient securely tied. Rarely did more than 30 seconds elapse between the stunning of the donor and the tying of the final knot in the skin of the recipient.[3]

Data on control rats. A total of 208 animals was born from the second (F_1) to the fifth (F_4) generation of the original 2 pairs of operative controls (animals subjected to the technique concerned in thymectomy without actual removal of the thymus gland). Of this number 183, or 87 per cent, survived the 70-day period of observation. As was pointed out in previous communications, the rate of growth and development in this special group of operative controls differed in no material way from the growth and development of the normal control series of the Philadelphia Institute for Medical Research. In figure 2 is shown the rate of growth of these controls as reflected by the average weight curve, and in table 1 is given the data on somatic development.

[3] The donor was then killed by chloroform.

RESULTS OF THYMUS IMPLANTATION IN SUCCESSIVE GENERATIONS OF THYMECTOMIZED RATS

Growth and development of test rats of the first generation (F_0). No effect on the growth and development of the 3 original pairs of thymectomized thymus-implant animals was observed. The average period between gestations was 47.5 days, as compared with 28.5 days in the operative controls, and 37 days in the control series of the Philadelphia Institute.

Second generation (F_1). A total of 93 rats was born in 12 litters to the 3 pairs of thymectomized thymus-implanted rats of the first generation (F_0). The litters varied in size from 3 to 11, with an average of 7.7 animals per litter. Of the total of 93 rats, 80, or 86 per cent, survived the 70-day period of observation.

In the second generation (F_1) thymectomy strain, a definite retardation in the rate of growth was noted (1, 2). In the offspring of the second generation (F_1) thymectomy thymus-implant strain, this retardation in growth was not only completely overcome, but the average growth of the operative controls was exceeded by a slight margin. Figure 2 shows the average growth curve of the entire group of 93 rats of the second generation (F_1), and table 2 the average weights of the entire group at 10-day intervals, together with the percentage in excess of the weights of the operative controls. Somatic development was slightly accelerated, the opening of the ears, eruption of the teeth, appearance of hair, opening of the eyes, descent of the testes, and opening of the vagina occurring on the early normal day, and occasionally earlier. In one litter, the upper incisors erupted on the seventh day, and the eyes opened on the 12th day.

Third generation (F_2). A total of 164 rats was born in 19 litters of the third generation (F_2). The litters varied in size from 4 to 13, with an average of 8.6 animals per litter. Of the total of 164 rats, 138, or 84 per cent, survived the 70-day period of observation.

Marked acceleration in the rate of growth was noted. Figure 2 shows the average growth curve of the 164 rats of the third generation (F_2), and table 2 the average weights of this group at 10-day intervals, together with the computed percentage over the weight of the operative controls. It was evident from a study of the average weight curve and the average weights at 10-day intervals that the test rats of the third generation (F_2) showed the greatest acceleration in growth during the first 6 weeks of life, and that this acceleration was greater than that shown by the test animals of the second generation (F_1).

Striking precocity was observed in the opening of the ears, the eruption of the teeth, the appearance of hair, the opening of the eyes, the descent of the testes and the opening of the vagina (table 1). In 2 litters the upper incisors erupted on the third day, in 2 others on the fourth day, in 2 on the fifth day, and in 4 on the sixth day. In one litter, the eyes opened on the seventh day, and in 4 on the eighth day (fig. 3). In none of the test rats was the opening of the eyes delayed beyond the 14th day.

Fourth generation (F_3). A total of 131 rats was born in 17 litters of the fourth generation (F_3). The litters varied in size from 5 to 9, with an average

of 7.7 animals per litter. Of the total of 131 rats, 115, or 88 per cent, survived the 70-day period of observation.

As figure 2 and table 2 show, the test rats of the fourth (F_3) generation showed considerable acceleration in growth, extending from birth through the 50th day, reaching a maximum of 21 per cent in excess of the operative controls at the age of 20 days. This precocity was even greater than that shown by the test rats of the third generation (F_2), exceeding it by an average of 8 per cent at 20 days, and 6 per cent at 30 days.

Somatic differentiation occurred earlier than in the preceding generations (table 1). In 5 litters, the upper incisors erupted on the fourth day, and in 3 litters on the fifth day. In 4 litters the eyes opened on the seventh day, and in 3 litters on the eighth day. In several instances, the test rats were seen climbing

TABLE 2. AVERAGE WEIGHT IN GRAMS AT 10-DAY INTERVALS OF SUCCESSIVE GENERATIONS OF THYMUS-IMPLANT-THYMECTOMY GROUP, AND PERCENTAGE OVER WEIGHT OF THE OPERATIVE CONTROLS

Age, Days	Operative Controls	F_1	% difference	F_2	% difference	F_3	% difference	F_4	% difference
Birth	4.9	4.9	0	4.9	0	5.2	6	5.2	6
10	15.0	15.6	4	17.2	15	17.6	17	18.0	20
20	28.5	30.5	7	32.1	13	34.6	21	34.6	21
30	49.1	54.6	11	55.2	12	58.0	18	58.9	20
40	68.6	75.7	10	77.0	12	78.9	15	81.0	18
50	93.5	94.5	1	97.1	4	102.9	10	104.8	12
60	113.3	115.4	1	117.5	4	122.4	8	123.7	8
70	135.3	137.5	1	138.7	3	140.7	4	141.4	5

vigorously over the walls of the cage, on the eighth and ninth days, and eating cracked corn from the food cups suspended in the cage. One litter was thymectomized and weaned successfully on the eighth day.

Fifth Generation (F_4). A total of 170 rats was born in 21 litters of the fifth (F_4) generation. The litters varied in size from 6 to 11, with an average of 8.1 animals per litter. Of the total of 170 rats, 112 or 66 per cent reached the end of the 70-day period of observation at the time of this writing, on February 1, 1937.

Again marked precocity in both growth and development was noted (fig. 2, table 1). The most rapid period of growth occurred during the first 50 days of life of the test rats, reaching a maximum of 21 per cent greater than the weight of the operative controls at the age of 20 days. In general, the average growth of the test rats of the fifth generation (F_4) was greater than that of the fourth generation (F_3), again indicating a cumulative effect in successive generations.

Precocity in somatic differentiation was as striking as the accelerated rate of growth of these animals. In 3 litters, the upper incisors erupted on the second day, in another 3 on the third day, and in 4 on the fourth day. In two litters, the eyes opened on the third day, in one litter on the fourth day, and in one litter on the fifth day. The increased activity of these rats was even more marked than that noted in the fourth generation (F_3).

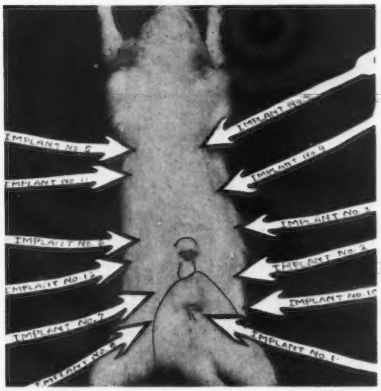

Fig. 1. Photograph of rat showing location of implants. Implant No. 1 made 1 week previous to the time photograph was made, may be readily seen. Subcutaneous pocket and ligature for implant No. 2 are also shown.

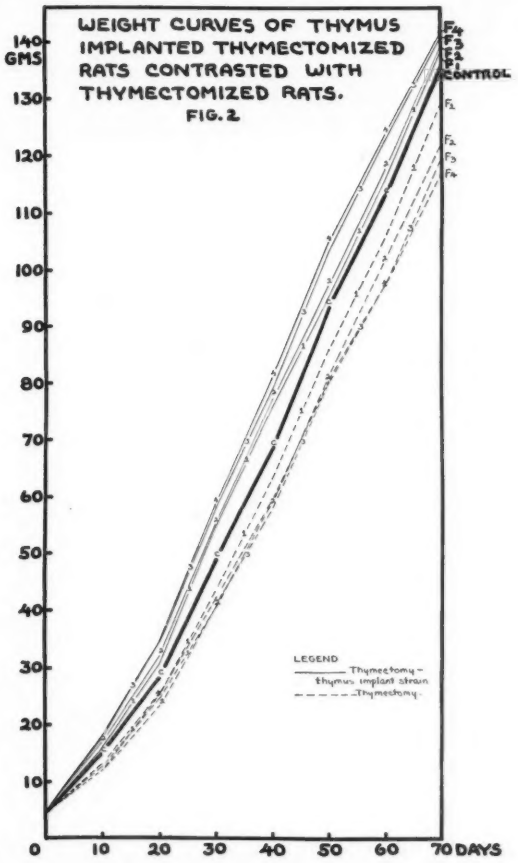

Fig. 2.

FATE OF IMPLANTS

A few of the implants in each animal sloughed, the remainder were readily palpable for about 10 days, but by the end of the second week could no longer be palpated. In the third generation (F_2) parent rats, implantations were made for a time at 5-day intervals, but it seemed as if this interfered with the normal activity of the animals, probably because of numerous painful areas on the

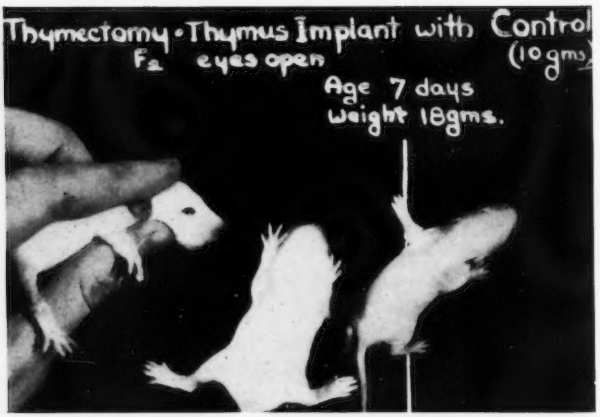

Fig. 3. Photograph showing acceleration in growth and development in third generation (F^2) thymectomy-thymus-implant strain.

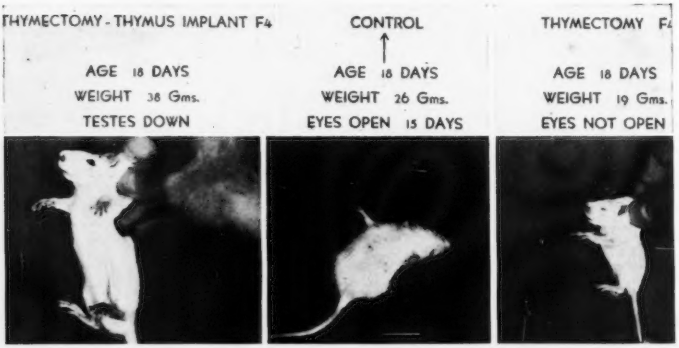

Fig. 4. Photographs showing over-correction of retardation in growth and development in thymectomy group of rats by means of substitution therapy with homologous thymus implants.

back, so weekly intervals were again followed throughout the experiment.

When the animals were sacrificed, areas of yellow pigmentation, about 1 cm. in diameter, were found subcutaneously in locations corresponding to the sites of implantation. These were sectioned and studied by Dr. J. H. Clark, pathologist to the Philadelphia General Hospital. He states that the areas of pigmentation were made up of circumscribed areas of reticular tissue resembling in every respect the tissue found in implanted normal rats; and that this reticular tissue could easily be thymus in origin, but that unless he had known before-

hand that these were thymus implants, he would not be justified in calling them thymus tissue. Low and high-power photomicrographs of a thymus implant removed from a killed animal 6 weeks after implantation are shown in another communication. Heterologous implants of calf thymus have been tried, but their use was discontinued early in the experiment because of the frequency of severe sloughs.

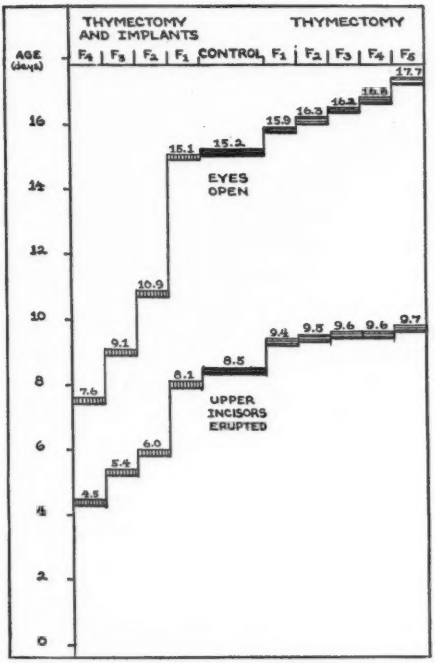

Fig. 5. Over-correction of retardation in somatic development occurring in the thymectomy test rats, by means of substitution therapy with homologous thymus implants.

DISCUSSION

With reference to the ability of frequent homologous thymus implants to overcome the retardation incident to thymectomy, it is of interest to compare the accelerated rate of growth and somatic differentiation in the offspring of implanted thymectomized parent rats with not only the normal observed in the operative controls, but also with the retarded growth and differentiation encountered in the untreated thymectomy strain.

Just as marked retardation in growth and development was observed in the fifth generation (F_4) thymectomy test strain (2), so marked acceleration in growth and development appeared in the fifth generation (F_4) thymectomy thymus-implant test strain. At the age of 30 days, the average weight of the thymectomy strain of animals was 42.2 gm., that of the operative controls 49.1 gm., and that of the thymectomy thymus-implant strain 58.9 gm. The thy-

mectomy animals were therefore 6.9 gm. or 14 per cent under, while the implant animals were 9.8 gm., or 20 per cent over the weight of their controls. Thus, by means of frequent homologous thymus gland implants, it was possible not only to overcome the 14 per cent retardation in growth incident to thymectomy, but to attain an excess of 20 per cent above the weight of the operative controls. Treatment therefore caused a 34 per cent increase over that of their thymectomized controls.

The acceleration in somatic differentiation was even more marked than the acceleration in growth. It is realized that the designation of the exact time of opening of the ears, the appearance of fur, and the descent of the testes depends to some extent upon the experience of the person who makes the observations. Of all the vital phenomena concerned in somatic differentiation, the eruption of one or more upper incisors, and the opening of the eyes are probably the most clearly defined changes noted in the development of the rat, and the personal equation is but little if at all concerned in the recognition of these phenomena. In this presentation, emphasis has been placed on these somatic changes. In the fifth generation (F_4), thymectomy test strain, the average time for the eruption of the upper incisors and the opening of the eyes was 9.6 and 16.8 respectively. In the implanted thymectomized test strain, these vital phenomena were observed much earlier, the teeth erupting and the eyes opening on an average of 4.5 and 7.6 days respectively (fig. 4, and 5). It would seem then that a single thymus gland implanted at weekly intervals until a total of 12 implants have been made is more than sufficient to correct the retardation in growth and development incident to thymectomy. At the present time, experiments are being conducted to determine how frequently implants must be made in order just to overcome these deficiencies.

SUMMARY

This investigation has been carried on for more than 2 years, and is based on observations of 772 animals in 5 generations, distributed as follows: operative controls 208, first generation (F_0) 6, second generation (F_1) 93, third generation (F_2) 164, fourth generation (F_3) 131, and fifth generation (F_4) 170.

With frequent homologous implants, the effects of thymectomy have been overcome in the second generation (F_1), and in succeeding generations there has occurred an acceleration in the rate of growth and development. This precocity was more striking than that described in another communication for the thymus-treated thymectomy strain, indicating that frequent homologous implants were more effective, in correcting the deficiencies following thymectomy, than heterologous thymus extract prepared from the thymi of calves, due possibly to the specificity of the glandular product involved.

Technical success in implanting numerous thymi appears to depend in part upon rapid transfer of the gland from their donor to the recipient, whereas biological effects depend upon the number of such implants and their homologous nature.

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NEGATIVE EFFECTS OF ENDOCRINE EXTRACTS ON THE THYMUS OF THE WHITE RAT[1]

FRANK NORMAN LOW
From the Department of Histology and Embryology, Cornell University
ITHACA, NEW YORK

The thymus of the white rat reaches the peak of its development at about the age of 85 days. The period between the time of weaning, at 21 days, and the initial thymus reduction was investigated in the following manner. The animals were kept in small cages in a warm cellar near the steam pipes of the building, where the temperature never got below 70°F. The sexes were segregated, and the animals fed special rat food marketed by the Wistar Institute. During the experiments, several in the colony suffered from snuffles and bronchial râles, but these ailments never lasted over a few days, and were not severe enough to cause any pathological changes save those mentioned in the following pages. The rats were weighed every third day, and a record kept of the weight gains to guard against inanition. Records were also kept of the general health of each animal. Any showing poor health not traceable to the treatments were discarded, and normal healthy controls were used in all experiments.

The extracts used in this investigation were anterior pituitary extract (supposed to contain alpha and beta cell hormones), Eli Lilly and Co., No. 198; ovarian residue extract (whole ovary without corpora lutea, supposed to contain theelin), Eli Lilly and Co. No. 196; desiccated thyroid substance, Eli Lilly and Co., No. 100; eschatin, adrenal cortex extract, Parke Davis and Co., and parathyroid extract, Eli Lilly and Co., unnumbered.

At the termination of the experiments, the animals were anaesthetized with chloroform or nembutal, and while the animal was still alive the thymus was removed and immediately fixed in either Zenker's fluid, or 10 per cent formalin. The tissue was fixed from 2 to 12 hours, according to the size of the piece. It was then dehydrated and imbedded in paraffin. The sections were cut 5 μ thick, and stained with either hematoxylin and eosin, eosin and methylene blue, or Mallory's tri-chrome stain. It was not considered necessary to make serial sections. A cross-section of the organ about half-way between the cephalic and caudal extremities was chosen as likely to be the most representative. This section always contained both lobes and abundant stroma between them.

EXPERIMENTAL PROCEDURE AND RESULTS

With the exception of the thyroid extract which was given orally, all of the extracts were administered in daily subcutaneous injections. In the case

[1] Abstract of a thesis presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

of the thyroid extract, the rat was deprived of liquids for 2 hours before the dose was to be given; the thyroid substance was mixed with milk or water, preferably the former and, given the animal in a small dish, it was readily taken. In the cases of the other rats, the experiments were conducted on a basis of constant daily doses, but with larger doses for each successive run.

Anterior pituitary extract (alpha and beta cell hormones). One cc. of the substance represented 1.2 gm. fresh pituitary substance. In the first series, 7 rats were treated for periods of 24 to 49 days, with a dilution of 1 part of extract to 18 parts of distilled water, 0.1 cc. being injected daily. In the second series, 4 rats were treated for 40 days with a dilution of 1 part of extract to 2 parts of sterile distilled water, .06 cc. being injected daily. In the third series, one rat was treated for 51 days, and another for 61 days, with the same dilution of extract as those in the second series, and with the same dosage. In the fourth series, a single rat was treated for 50 days with the same dilution of extract as in the second and third series, 0.1 cc. being injected daily. In the fifth series, one rat was treated for 30 days with undiluted extract, 0.15 cc. being injected daily.

Histologically, the thymi of these animals presented a normal picture. There was nothing consistent in the appearance of the tissue that was definitely traceable to the effects of the treatment.

Ovarian residue extract (theelin). One cc. of the substance represented 2 gm. fresh ovarian substance without corpora lutea. In the sixth series, 8 rats were treated for periods of 17 to 44 days with a dilution of 1 part of extract to 4 parts of sterile distilled water, 0.05 cc. being injected daily. In the seventh series, 4 rats were treated for periods of 37 to 50 days with the same dilution as in the sixth series, 0.1 cc. being injected daily. In the eighth series, 2 rats were treated for a period of 30 days with undiluted extract, 0.15 cc. being injected daily.

The results of these experiments were negative. The thymus showed no histological changes which were consistent, or which could be related to the treatment.

Desiccated thyroid substance. In the ninth series, 6 rats were treated for periods of 19 to 35 days with 0.25 gr. U.S.P. thyroid daily. In the 10th series, a single rat was treated with 1 gr. U.S.P. thyroid per day for a period of 1 month.

All the rats demonstrated symptoms typical of hyperthyroidism, especially increased appetite and shortness of breath. Upon histological examination, the thymi seemed to be normal except for a very dense cortex which appeared thicker than usual. Upon actual count, it was found that the number of cortical lymphocytes had been materially increased. Since rapid action of the fixing fluid was desirable, it was considered impractical to weigh the thymi; thus there was no record of gross increase in size. With the aid of a calibrated ocular micrometer, accurate counts of the numbers of cortical lymphocytes per 1500 square micra of a five micra section were made. In the treated animals, this number varied from 102 to 116 against a count of 72 to 95 in the controls. The lymphocytes seemed to be especially thick at the periphery of

the lobules and, to avoid inaccuracy, the count was taken about midway between the periphery and the medulla.

The rat treated in the 10th series was not vigorous at the end of the treatment, being in fact emaciated to a degree which would ordinarily have produced an accidental involution. However, the lymphocyte count was the same as in the other treated animals, and the thymus appeared healthy.

Eschatin—Adrenal cortex extract. One cc. of the material represented 40 gm. of fresh cortical substance. In the 11th series, 3 rats were treated for 23 to 33 days with undiluted extract, 0.03 cc. being injected daily. In the 12th series, 3 rats were treated with undiluted extract, 0.20 cc. being injected daily for a period of 1 month. The results of these experiments were negative; all of the animals possessed normal thymi.

Parathyroid extract—Each cc. of the extract contained 100 U. In the 13th series 6 rats were treated for 28 to 37 days with 4 to 5 U. daily.

In the 14th series, a single rat was treated with 4 to 5 U. daily for 42 days.

The thymi of the rats of the 13th series were normal. The rat in the 14th series fell ill with a respiratory ailment which seemed to differ from the same condition in the control animals. During the course of the treatment, it became extremely emaciated, and the thymus showed extreme involution. However, this could not be attributed entirely to the effects of the treatment, as a rat subsequently treated with a much larger amount (20 U. daily) possessed a perfectly normal thymus at the end of the experiment.

Combinations of extracts. Various combinations of extracts were used in an attempt to bring about some change in thymic structure. The combinations were: adrenal and ovary, adrenal and thyroid, adrenal, thyroid, and ovary, thyroid and ovary.

In the cases of the first 3 treatments, listed above, the results were negative. In the case of the rats treated with thyroid and ovary, all of the animals fell so ill from the effects of the treatment that they had to be sacrificed before the end of the experiments. The ailment was respiratory in nature. Consequently, additional treatments with the same extracts were undertaken. One of the animals died in convulsions on the sixth day of treatment, and possessed the only normal thymus of the entire group. The thymi of the first rats subjected to this treatment all showed definite signs of involution. In 2 of the 3, there was wholesale destruction of lymphocytes in situ. Signs of phagocytic action on the part of the reticular cells were evident. The thymus of another rat, treated for 18 days, showed some signs of involution, there being very poor distinction between the cortex and the medulla, coupled with subnormal numbers of lymphocytes. The author concludes that the treatment in some way rendered the rats more susceptible to respiratory disorders, and that the involution was traceable to the infection and therefore secondary to the treatment.

DISCUSSION

The negative results reported here seem to indicate that the thymus is extremely unreactive to direct endocrine stimulation. However, it must be remembered that the materials used for these treatments were not the pure

form of the substances secreted by the glands, but merely extracts believed to contain the active principle. The amount necessary to cause changes in thymic structure has been little more than a matter of conjecture in all of the cases, except that of thyroid substance where the toxic dose for rats is known.

The increase in the number of cortical lymphocytes in the thymi of the thyroid-fed rats is about what would be expected in view of the fact that an excess of thyroid substance causes hypertrophy of the thymus. Such thymus reduction as has been accomplished with ovarian endocrines has been traceable to a substance extracted from the urine of pregnant mares. This is doubtless the same principle that is responsible for the involution of pregnancy. In the extract used, there was probably none of this substance present, since the material was prepared from ovary without corpora lutea.

The extracts of the anterior pituitary gland and the adrenal cortex were prepared without reference to action on the thymus, and it is possible that the elements reputed to act on the thymus in acromegaly and Addison's disease were removed in the course of preparation. There is also the possibility that the action in the cases of these diseases is brought about indirectly by a complex of reactions which the artificial stimulation has failed to set up.

The evidence in favor of the action of parathyroid extract on the thymus is very slight. The author believes that there is no direct evidence connecting the two, and that any involution would be due to a general cachectic state of the animal, brought about by treatment of too much parathyroid extract.

The negative results of this series of experiments are comprehensible if the thymus is assumed to be affected only by conditions altering the general body metabolism, improving or depressing it. Much has been written about the suspected relationships between the thymus and the endocrine glands, but it would not be very difficult to imagine that the thymic changes in endocrine diseases are caused by the effect of the disease on the general body metabolism. The direct excitation of the thymus by the thyroid is the only exception.

In conclusion, it may be said that the results reported here need not serve to negate the indications of inter-relationships afforded by natural pathology and extirpation experiments. It is possible that further investigations with larger amounts of extract and more reactive animals will throw light on the situation.

SUMMARY

The action of anterior pituitary extract (alpha and beta cell hormones) on the thymus is negative, as is the action of ovarian residue extract (theelin) on the thymus. Desiccated thyroid substance, when administered orally in proper amounts, increases the number of cortical lymphocytes in the thymus. The action of eschatin (adrenal cortex extract) on the thymus is negative, as is the action of parathyroid extract on the thymus. Rats treated with a combination of thyroid and ovary preparations are in some way rendered susceptible to infections, which in turn cause the thymus to undergo involution.

A STUDY OF NATURAL GROWTH AND OSSIFICATION IN HEREDITARY DWARF MICE[1]

E. BOETTIGER AND C. M. OSBORN
From the Biological Laboratories, Harvard University
CAMBRIDGE, MASSACHUSETTS

Hereditary dwarfism in mice has interested geneticists and endocrinologists since 1929, when Snell (1) reported the mutation which behaved as a true Mendelian recessive. However, the dwarfs, being entirely sterile, could be produced only from heterozygous parents. Snell (2) also described their thyroid deficiency, and obtained restorative effects with a thyroid-stimulating extract made from the anterior pituitary which produced body growth and caused fertility in one male. Similar results were obtained by feeding powdered thyroid.

The endocrine problem presented by these animals was further outlined and studied by Smith and MacDowell (3, 4), who concluded that no eosinophiles were present in the anterior pituitary, and observed that implants of these pituitaries gave no growth response. Snell (2), MacDowell (5) and MacDowell, Laanes and Smith (6) reported that feeding desiccated thyroid resulted in marked gains in body weight but abnormal growth (fatty subcutaneous and intraperitoneal deposits). Kemp (7), using a growth-stimulating preparation, extracted from the pituitary, reported growth in several animals without proportional differentiation of organs. Dawson (10) reported that skeletal differentiation in dwarfs occurred in normal sequence, but at a greatly retarded rate. Diaphyso-epiphyseal union was not suppressed in a group of epiphyses normally uniting earliest in the mammal.

Data collected on a considerable number of dwarfs in our colony indicate that a hitherto unappreciated variability exists in both the rate and the extent of their differentiation and growth. In order to establish a standard for experimental work, we have adopted here a purely descriptive approach. In this attempt to determine for a dwarf what may be considered natural development at a given time in life, we have considered body weight, body length, tail length and progressive ossification.

MATERIALS AND METHODS

This colony was developed from the original Bussey stock used by Snell. Dwarfs were produced in two ways, from heterozygous parents and by mating dwarf males, made fertile with pituitary extracts, to heterozygous females. Litters were born and reared in cages containing both parents. As soon as the dwarfs could be distinguished (4 to 10 days of age) enough of the normal litter mates were killed to reduce the total litter number to 4 or 5. The

[1] We wish to express our appreciation to Professor A. B. Dawson for his helpful suggestions.

remaining normals were removed between 2 and 3 weeks of age. The dwarfs were allowed to stay throughout most of the suckling period of the successive litter and were then removed, when 30 to 40 days old, to smaller cages. The temperature in the animal rooms was maintained at 72°F. and the humidity at 40 per cent saturation. Water and Purina dog-chow were always available in every cage, and fresh lettuce was given weekly to supplement the diet. Data comparable to those collected on dwarfs were also recorded on a limited but fairly representative number of normal litter mates. The method of Sumner (9) used subsequently by Greene and Fekrete (11), was used in

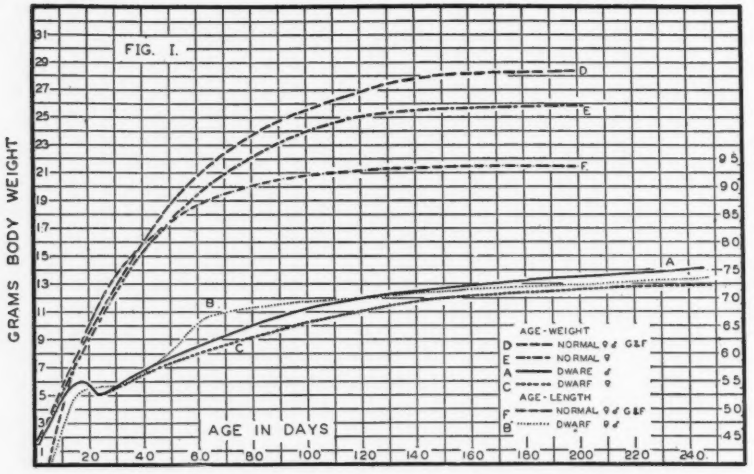


Fig. 1. Curves *A* and *C* are weight-growth curves of male and female dwarfs, respectively. *D* and *E* are weight-growth curves of normal animals, *D* (both males and females) from the data of Greene and Fekrete (11), and *E* from data on females of our colony. Curves *B* and *F* are length-growth curves of dwarf and normal mice, respectively. Each plotted point on all curves represents the average of all the weights or lengths on animals of a given age. Since more data were collected at some ages than at others, the value of each point was weighted as the curve was drawn.

recording body and tail lengths; and ossification was studied on skeletons stained by the technic of Dawson (8).

OBSERVATIONS

Body weight as a measure of growth. Curve *A* (fig. 1) represents 1149 body weights of about 150 male dwarfs plotted against age in days. Curve *C* of the same figure represents 1451 body weights of about 225 female dwarfs. *E* is the growth curve of the normal virgin female litter mates. Over 200 weights of about 40 animals were used in building this curve. *D* is a growth curve drawn from the data of Greene and Fekrete (11), and includes males and females. As may be seen, curves *E* and *D* are very similar in shape, although the adult normals in our colony are somewhat lighter.

Curves *A* and *C* are essentially alike, except that the male dwarf grows somewhat faster after 25 days of age, and reaches a slightly higher maximum. During the first 25 days of life, the curves are almost identical. The remarkable part of the dwarf curve is its unique shape between 17 and 30 days of

age. When about 17 days old, the dwarf not only stops growing, but actually begins to lose body weight. This loss varies in individuals from 5 to 35 per cent. Most animals losing 30 to 35 per cent die, but the occassional ones that survive cannot be distinguished later from other dwarfs.

Some idea of the variation among dwarfs during and subsequent to this critical period may be gained from figure 2, where individual growth curves have been plotted. The dwarfs follow the normal curve for the first 10 to 12 days, and then drop slightly behind. Individual curves show cases where dwarfs could not be distinguished from normal litter mates at 16 days on the

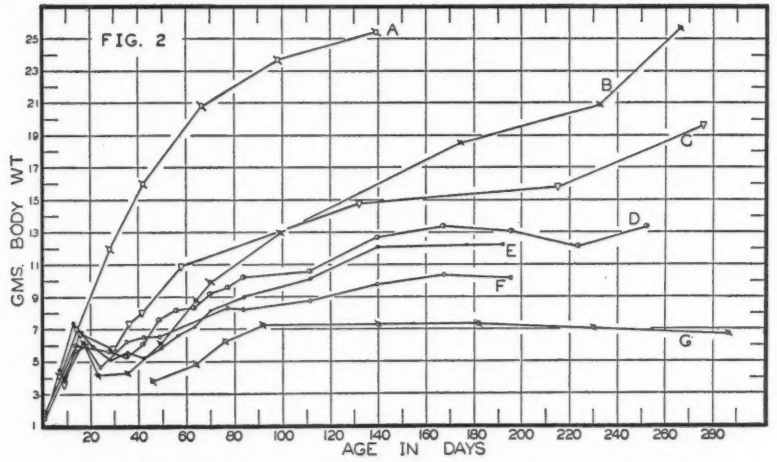


Fig. 2. Individual body-weight growth curves of dwarf mice showing average (*D* and *E*) and extreme (*B* and *G*) examples of the body-weight/age relationships.

basis of weight alone. However, other dwarf characteristics as described by Snell (1) distinguish them long before weight is a dependable criterion. During the latter part of the critical period, the dwarf begins to gain weight. When percentage weight increases are calculated for the period from 25 to 140 days, it is found that the amount of growth relative to the body size at 25 days is almost identical for dwarf and normal mice. When the dwarf is 140 days old, it has gained 134 per cent over its weight at 25 days. Calculated from data of Greene and Fekrete (11) normal mice gain 142 per cent during the same period. A similar comparison holds for body elongation during this time. In the dwarf, the body length increases 31 per cent, whereas in the normal the increase is 33 per cent.

Not only is the total percentage weight increase similar in dwarf and normal mice over this period, but the distribution of the increase is approximately the same. This is shown in table 1, where the percentage increases during weekly intervals have been calculated.

Unfortunately, body weight is not a good criterion of growth throughout the entire life span of the dwarf. It is evident from the curves that both dwarf and normal mice reach their plateaus at 140 to 150 days of age. After that

time, the dwarf may vary greatly in weight, due to a tendency to accumulate large deposits of subcutaneous and intraperitoneal fat. This is true of so many of the animals that, when weights are averaged, it has tended to effect a continuous rise in the latter part of the weight curve. However, body-length increases during this period indicate that the skeleton of the dwarf continues to grow after the time when the normal has reached its maximal adult length. The late closure, at 6 to 8 months in the dwarf (10), of epiphyses uniting at 6 to 8 weeks in the normal could account for the skeletal growth continuing late in the dwarf's life (curve *B*, fig. 1).

Body length as an indicator of growth. As a means of avoiding weight error due to fatty deposits, consideration was given to body-length measure-

TABLE 1. PERCENTAGE WEIGHT INCREASES AT WEEKLY INTERVALS

Age in Days	Percentage Weight Increase			
	Normal[1] Males and Females	Dwarf Males and Females	Dwarf Females	Normal Females
7 to 14	94	58	54	75
14 to 21	33	−2	−3	34
21 to 28	23	−2	0	28
28 to 35	19	14	13	19
35 to 42	12	10	10	12
42 to 49	10	8	7	8
49 to 56	10	7	7	8
56 to 63	6	6	6	6
63 to 70	6	5	5	5
70 to 77	5	6	4	4
77 to 84	3	4	3	3
84 to 91	3	4	4	3
91 to 98	2	4	4	2
98 to 105	2	3	3	2
105 to 112	2	3	3	2
112 to 119	1	2	3	1
119 to 126	1	2	2	1
126 to 133	1	2	3	1
133 to 140	1	1	2	1

[1] Calculated from the data of Greene and Fekrete (11).

ments. These are plotted in figure 1 on the right vertical axis against the age axis used in the previous curves. Curve *B* represents the body lengths of over 160 dwarfs, and may be compared with the body-length curve (*F*) of the normal mice.[2] In many respects, the 2 length curves relative to the body-weight curves are similar. During the dwarf's critical period, length increases are slight, as skeletal growth is almost at a standstill. This is not surprising in view of the fact that the animal is actually losing weight during the first half of the period. Of special interest is the sudden acceleration in skeletal lengthening, which occurs between 40 and 70 days. During this period, the length curve ascends above the weight curves, indicating that the skeleton temporarily is disproportionately large. A similar but less extreme condition is expressed in the normal mouse curve, *F*. Here the length stays above the weight curve

[2] From the data of Greene and Fekrete (11).

between 18 and 40 days. Both length curves plateau at 140 to 150 days, coinciding in time with the flattening of the weight curves. Relative to the weight curve plateaus of both dwarfs and normals, the respective length curves fall quite differently. In the dwarf, the weight and length curves almost coincide after 120 days, whereas in the normal the length falls significantly below the weight at 60 days. This indicates that normals after 60 days of age are much heavier per unit length than dwarfs. In weight per unit length, the dwarf is more nearly comparable to the normal of the same weight than to one of the same age.

Body-tail proportions in the dwarf. We have been interested in body proportions as well as absolute body size. The body-length/tail-length ratio

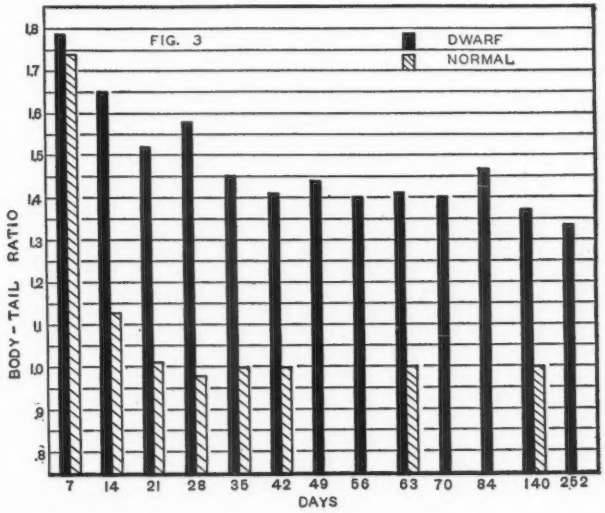


Fig. 3. The body-length/tail-length ratios of dwarf and normal mice are represented on the ordinate; the abscissa represents age in days. Data on normal animals are not included at all of the ages listed, because it may be seen that the ratio falls to about 1.0 at 21 days, and subsequently remains constant. The ratio for the dwarf is permanently relatively high.

has been studied rather extensively by geneticists. It is commonly known that mice are short-tailed when born. Snell (1) noticed that the dwarf tail was normally short at birth and, although the normal tail lengthened rapidly, the dwarf tail was much retarded in growth. This characteristic has been used as a distinguishing feature of the dwarf. However, Snell believed that later in life the ears and tail developed sufficiently to make the proportions those of the normal adult. We have taken appropriate measurements of over 160 dwarfs, and have plotted the body-length/tail-length ratio against age, in figure 3. The ratios of the normals are calculated from data given by Greene and Fekrete (11). This graphic representation shows that our dwarfs are permanently short-tailed, the ratio remaining considerably above the value 1.00, to which the normal descends by 21 days.

Ossification. The time of appearance of ossification centers in the normal

mouse was studied by Johnson (12), and occurs with remarkable regularity in all animals kept under normal conditions. As an index of the rate of differentiation, skeletal development furnishes one of the most easily determined criteria.

The skeletons of 75 dwarfs between the ages of 7 and 84 days were studied for the presence or absence of all the centers of the tibia, fibula, humerus, ulna and radius appearing after birth, with the exception of the greater tuberosities of the humerus and tibia and the lateral condyle of the humerus. On the basis of the appearance of secondary ossification centers, Dawson (10) has stated that the 6 to 8-week-old dwarf is comparable to a normal 7 to 9 days of age. In animals typical of our standard growth curve,

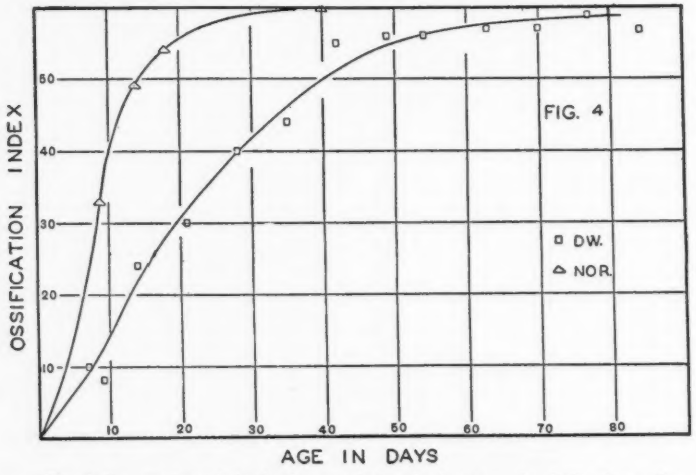


Fig. 4. A comparison of the development during the first 12 weeks of life of secondary ossification centers in normal and in dwarf mice. Twenty centers are represented and each evaluated 1, 2 or 3, according as they were small, medium or large. Thus, an animal possessing 20 large centers would be rated 60 on the graph.

we find the 14 and 15-day epiphyseal centers differentiated from 28 to 35 days of age, and an 18-day center (proximal fibula) is present in the dwarf by 42 days. The acromion of the scapula, appearing in the normal at 35 days, may be present in the 63-day-old dwarf, but is usually absent until 12 to 14 weeks. In the dwarf, a wide range of variation in the extent of skeletal differentiation parallels the variation in growth.

The retardation of skeletal development may be traced back to early life. A study of two 7-day dwarfs shows normal 7-day centers present in 1 case, and normal 5½-day centers in the other. No 9-day dwarf has been found to have normal 9-day centers. Although dwarfism may not be expressed in ossification before the seventh day, the degree of differentiation at this time is by no means constant. In the recognition of dwarfs, our most reliable criterion has been the shortened head, resulting from the failure of the cartilage bones at the base of the skull to elongate in normal fashion. The blunt nose and short tail

are the earliest dwarf characteristics to be noted, and are both the result of retarded skeletal development.

As a means of comparing the rates of skeletal differentiation in dwarfs and normals, we have set up an arbitrary ossification index. This index was obtained by assigning a value 1, 2 or 3 to each of the 20 centers studied, according to whether they were small, medium or large. These values were then added for each animal. The average index for different ages is plotted against time in figure 4, showing graphically how ossification lags in the dwarf. Both curves have essentially the same shape. Although there is no break in the curve during the critical period, there may be a definite slowing up of differentiation not obvious from our method of analysis. However, new centers do appear and continue to grow during this period.

The growth, as well as the differentiation of the bones, is shown in figure 5, and the weights and body lengths of the animals used for these photographs are given in the table in the legend to figures 5 to 10. We have chosen animals representative of our average curves. Compared with the normal of the same age, the 9-day dwarf is considerably retarded. The growth and differentiation of centers progresses from 9 to 14 days in both the dwarf and normal. At about 17 days, the critical period begins, and not until 35 days is skeletal growth resumed at an appreciable rate. The 35-day animal of figure 5 is slightly below the average for this age, and so the more rapid growth of the bones between 35 and 63 days is somewhat overemphasized; its reality, however, is none the less striking, and can be seen by tracing the increase in body length over the same period (fig. 1). The normal shows a steady and rapid increase in bone length and differentiation up to 63 days; subsequently, the growth rate is much reduced. Likewise, the dwarf after 63 days adds little to its skeletal dimensions. The size of bones in the average adult dwarf is shown in figure 6, and is compared with a normal 76 weeks. In shape and proportion, the dwarf bones are very comparable to those of the normal, whereas the lengths are from 70 to 75 per cent normal.

DISCUSSION

That dwarfism appears abruptly about the 14th day of life was stated by Snell (1) and confirmed by Smith and MacDowell (3). We have occasionally been able in our colony to pick out dwarfs by the fourth or fifth day, and almost always by the tenth. The majority of dwarfs are readily distinguished at 7 to 9 days of age, some days before weight differences are evident. The statement also appears in the early work on the dwarf that these mice grow scarcely at all after 14 days of age. Our growth curves fail to indicate this cessation, but show rather a definite growth in both weight and length until about 140 days.

In figure 10 are 4 dwarfs ranging in age from 20 days (animal *1*) to 18 months (animal *5*), examples indicating the growth which dwarfs may normally attain. These photographs and those of the bones, together with the curves of figures 1 and 2, afford concrete evidence of growth in the dwarf mouse. Animal *5* is not included as typical, but shows how heavy an un-

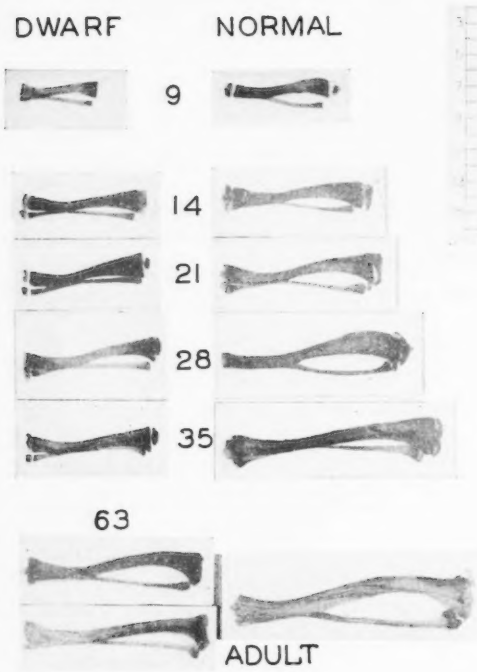


FIG. 5

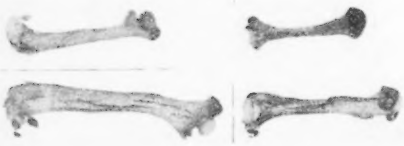
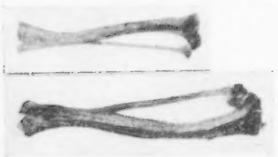


FIG. 6



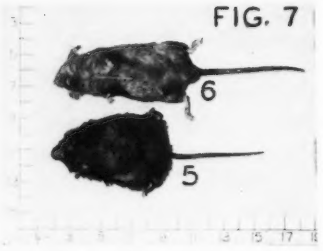


FIG. 7

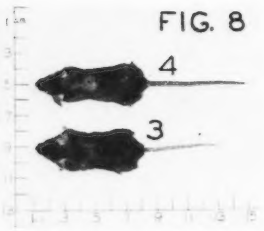


FIG. 8

FIG. 9

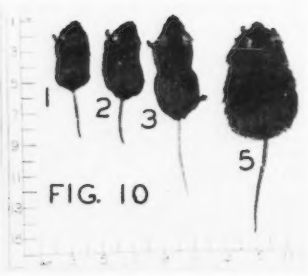


FIG. 10

Fig. 5. A chronological series of tibiae and fibulae of dwarf and normal mice showing the relative growth and differentiation of these two bones. Twice natural size.

Fig. 6. A comparison of an adult dwarf and a normal femur, humerus tibia and fibula. Twice natural size.

Fig. 7, 8, 9, 10. Four dwarfs (animals *1, 2, 3,* and *5*) and 2 normals (animals *4* and *6*) variously grouped to show relative size, body proportions, tail length, and fatty accumulations at different ages. See also tables 2 and 3.

treated dwarf may become (34 gm). Animal 3, a typical adult dwarf on the plateau of its growth curve, has not accumulated abnormal deposits of fat. With the exception of the short tail (fig. 3), the body proportions of dwarfs and normals of the same weight are quite similar (fig. 8).

The most striking aspect of dwarf growth to us is the existence of a critical period during which the average animal loses and then regains about 16 per cent of its body weight. This period coincides roughly with weaning time. That the explanation is not simply a lack of food is indicated by the presence of reasonable amounts in the digestive tract at autopsy. This is not proof that the dwarf is getting sufficient nourishment, however, for the animal may be unable to assimilate or absorb its food. The failing mechanism must

TABLE 2. DATA ON ANIMALS WHOSE BONES ARE PHOTOGRAPHED IN FIGURE 5

Age, days	Dwarf		Normal	
	Body Wt.	Body Length	Body Wt.	Body Length
9	3.9 gm.	45.5 mm.	5.7 gm.	51.1 mm.
14	5.3 "	52.0 "		
21	5.5 "	53.7 "	9.3 "	66.1 "
28	4.4 "	54.7 "	12.6 "	77.3 "
35	5.1 "	53.4 "		
63	10.3 "	68.4 "		
adult	14.0 "	75.5 "		

TABLE 3. DATA ON ANIMALS SHOWN IN FIGURES 7, 8, 9, 10

Animal	Age, days	Body Wt.	Body Length
1 Dwarf female	20	5.5 gm.	53 mm.
2 Dwarf male	42	8.0 "	60 "
3 Dwarf male	275	14.0 "	75 "
4 Normal male	35	14.1 "	76 "
5 Dwarf female	500	34.0 "	77 "
6 Normal male	350	33.1 "	95 "

be temporary in its effect, in view of the fact that once the critical period is past the animal is healthy, grows considerably, and approaches a normal life span. At weaning, the growth rate of normal mice slows down, but there is never an absolute loss in weight.

From the lowest point in the critical period, the dwarf begins to gain the weight lost, and continued growth is maintained. The mechanism of this growth may be the same as in the normal during this time. Table 1 shows that the percentage increases in length and weight from week to week, as well as for the whole period, are almost identical. It is as if the dwarf omits the period of most rapid growth in the normal, and then resumes growth when it is able to build tissue as efficiently as the normal of the same age.

Whether it is more legitimate to compare dwarfs and normals on the basis of age or of weight is questionable. The weight of the dwarf per unit body-length is similar to the normal of the same weight but far below the normal of the same age (fig. 1). Figure 8, shows that general body proportions of dwarfs and normals of the same weight are very similar, although the normal

is only 35 days of age and the dwarf over 140. The tail length of the dwarf, however, is similar to that of a normal younger than 35 days. Skeletal differentiation is much more advanced in dwarfs when they are compared with normals of the same weight, showing that differentiation is to a certain extent independent of growth.

Growth has been associated with a specific growth hormone secreted by the pituitary. Riddle (13) on the basis of evidence obtained from dwarf mice, has questioned the existence of any specific growth hormone secreted by the pituitary. It is true that some growth has been produced in dwarf mice in a variety of ways by various workers. Snell (2) produced very heavy and abnormally fat animals with injections of a pituitary thyrotropic preparation and the feeding of desiccated thyroid. No mention was made of increase in body length. The pituitary preparation improved the thyroid picture and increased general activity. MacDowell (5) confirmed Snell (2) and added that the thyroid treatment did not change the pituitary. By implanting fresh rat pituitaries into dwarfs, Smith and MacDowell (3) produced larger animals and repaired other endocrine deficiencies. However, when these investigators (4) implanted dwarf pituitaries into dwarfs, they obtained no growth. They interpreted this to mean that the growth hormone was either entirely lacking, or else suppressed in the dwarf pituitary. It is conceivable, however, that a specific growth hormone could be present in the dwarf pituitary, yet incapable of expression in a system deficient in secretions of the thyroids, adrenals and other glands.[3] Up to the present, the best growth has been obtained with pituitary implants, injections of relatively impure preparations, or a combination of 2 or more hormones. Bates, Laanes and Riddle (14) injected dwarfs with a variety of commercial growth hormone preparations and also combinations of prolactin and thyrotropic substance. Desiccated thyroid was fed to another group of dwarfs. They used animals 33 to 150 days old, which, as our curves indicate, is the time of most rapid natural growth in the dwarf. At the end of the 33-day period of injection, their weight increases were small (similar to natural increases shown in certain 33-day periods in our standard growth curve for the dwarf, fig. 1), when thyrotropic or prolactin were injected singly, but much greater when the 2 were given together. The greatest weight increases usually followed growth hormone injections. However, significant increases followed thyroid feeding. Investigators have consistently reported excess fatty accumulations in thyroid treated dwarfs and in animals injected with thyroid stimulating preparations. This seems inconsistent with hyperthyroid effects in mammalian physiology.

Recent work on hypophysectomized rats and dwarf mice has shown that the rôle of the pituitary in growth is more complex than was previously appreciated. Responses in dwarfs to growth preparations should not be compared with effects of the same preparations used in hypophysectomized animals. Furthermore, attention must be paid to the particular dwarfs used, and

[3] Benedict and Lee (15) reported that the basal heat production of dwarf mice (body wt. 8 gm.) was 81% that of normal albino mice per unit of weight and 60%, per unit of surface area.

an appreciation of the variability normally found must enter into the interpretation of the results.

SUMMARY

The growth of hereditary dwarf mice follows a curve peculiar to these animals. The fundamental growth differences between dwarfs and normals are apparently limited to differences in development between 17 and 35 days of age. During this time the normal grows very steadily and rapidly, but the dwarf experiences a critical period of retarded skeletal elongation and loses about 16 per cent of its weight. However, new ossification centers do appear during this time. Although the critical period coincides with weaning, this is probably not the direct cause.

Growth plateaus at 140 to 150 days in dwarf mice as in normals. Most dwarfs may be distinguished from normal litter mates at 7 to 9 days of age. Dwarfs are permanently short-tailed in contrast to normals.

Much greater variation in weight and general activity is exhibited by dwarfs than by normal mice. Dwarfs and normals between 25 and 140 days of age have almost identical percentage growth increases (weight and length). Adult dwarfs are lighter per unit body-length than normal adult mice. The length of the critical period and the amount of weight lost seem to bear no direct relation to the maximum growth attainable. Male dwarfs grow somewhat faster than females, and attain slightly heavier body weights.

Skeletal growth, as manifested by body-length increase, is most rapid between 40 and 70 days of age. Skeletal differentiation occurs in normal sequence, but is retarded in dwarfs as compared with normals. At 70 to 80 days, the dwarf shows an epiphyseal development comparable to that of normal animals 25 to 30 days old.

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AUTOTRANSPLANTATION AND REGENERATION OF THE ADRENAL GLAND

DWIGHT J. INGLE AND GEORGE M. HIGGINS
From the Division of Biochemistry and the Division of Experimental Medicine, The Mayo Foundation
ROCHESTER, MINNESOTA

It is well known that a functional graft of an adrenal gland is a mass of regenerated cortical tissue (1-7). Medullary tissue does not regenerate, although Wyman (8) found chromaffine tissue, identified by appropriate stains, in two cases of cortical transplants. Neither the extent of degeneration of an adrenal graft nor the source of regenerated cortex has ever been carefully studied. Pencharz, Olmsted and Giragossintz (6) transplanted adrenal cortex to the ovaries of rats, and included some illustrative data on degeneration and the cortical regeneration which followed.

Wyman and Walker (9) have shown that removal of the capsule from the gland was unfavorable to its successful transplantation. Wyman and tum Suden (10) showed that the size of regenerated cortical fragments depended on the number of fragments which survived after grafting. According to them, if 4 fragments were grafted to pockets in the abdominal musculature and 2 failed to survive, the regenerated fragments would be larger than any one fragment would have been if all 4 had survived (10). Some inhibitory mechanism, within the organism, appeared to limit the size of the regenerating fragments.

Since these earlier studies were concerned more with the physiologic activities of the restored graft than with its cytology, we undertook to study the process of cortical regeneration. If the capsule is essential for successful transplantation (9) does cortical regeneration depend on the capsule? Will the enucleated portion of the gland survive and regenerate when grafted without the capsule, or will the capsule, when grafted alone, cause regeneration of a gland? How does the presence of one intact gland affect the regeneration of a singly transplanted gland?

EXPERIMENTAL PROCEDURE

The following series of grafting experiments were carried out on adult, healthy, female rats, weighing from 175 to 200 gm. *1)* Both adrenal glands were removed from each of 30 rats and grafted to the ovaries. (Bilateral autotransplantation of both entire adrenals.) *2)* One adrenal gland was removed from each of 30, and grafted to an ovary, whereas the other gland was left in place. (Unilateral autotransplantation of one entire adrenal.)

3) Both adrenal glands were removed from each of 20 rats. The tip of

each gland was cut off, and the cortex and medulla were gently expressed from the capsule. The enucleated glands, thus expressed, were discarded and the capsules sutured to the ovaries. (Bilateral autotransplantation of the capsules.) *4)* One adrenal gland was removed from each of 20 rats, the nucleus expressed from each, and the capsule sutured to the ovary. The other gland was left in place. (Unilateral autotransplantation of the capsule.)

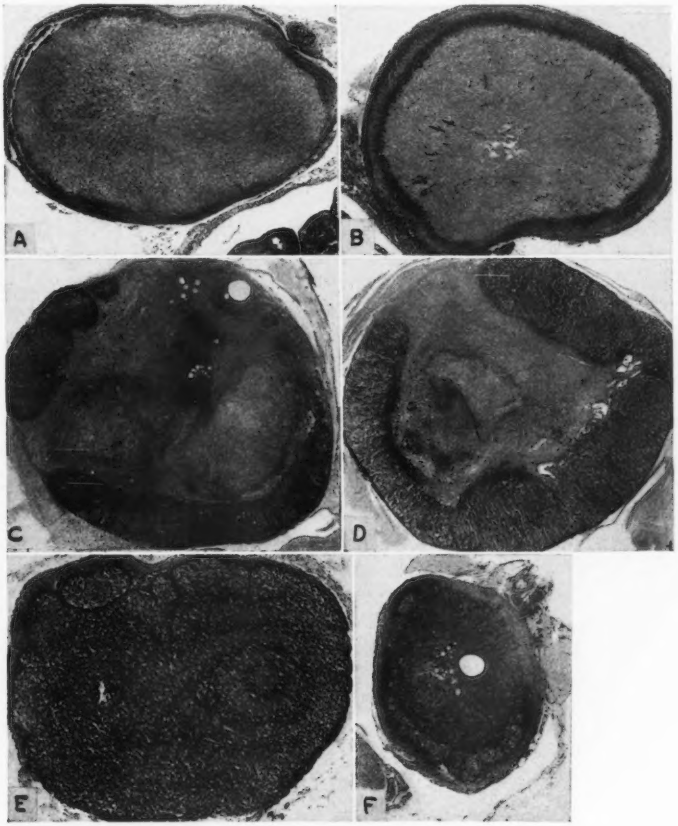

Fig. 1. Photomicrographs of adrenal glands grafted, entire, to ovaries: *A*, 48 hr. after bilaterial autotransplantation; *B*, 72 hr.; *C*, 2 weeks; *D*, 3 weeks after transplantation. (All H. & E. x15).

E, Regenerated cortical tissue in a gland 6 weeks after bilateral autotransplantation of adrenals, entire, to ovaries. x23.

F, Unilateral graft of adrenal 6 weeks after transplantation, entire, to ovary. x19.

5) Both adrenal glands were removed from each of 20 rats. The capsules were freed, in the manner described above, and discarded, and the enucleated portions sutured to the ovaries. (Bilateral autotransplantation of the enucleated adrenals.) *6)* One adrenal gland was removed from each of 20 rats. The capsule was removed and discarded, and the enucleated portion of the gland

sutured to the ovary. (Unilateral autotransplantation of the enucleated adrenal.)

In the removal of the capsule from the gland, small portions of the zona glomerulosa remained adherent to the capsule. In consideration of the results on regeneration, when the enucleated capsule had been sutured to the ovary, as described below, this fact must be kept in mind.

All animals were given maintenance doses of cortin for 10 days following operation. Animals were killed at intervals following transplantation, and the grafts removed, fixed and stained with hemotoxylin and eosin; they were also stained for fat and medullary tissue.

RESULTS

Bilateral autotransplantation of both entire adrenals. When both adrenal glands, entire, were transplanted to the ovaries, degeneration of the glands followed immediately. Necrosis began in the center of the organ and extended

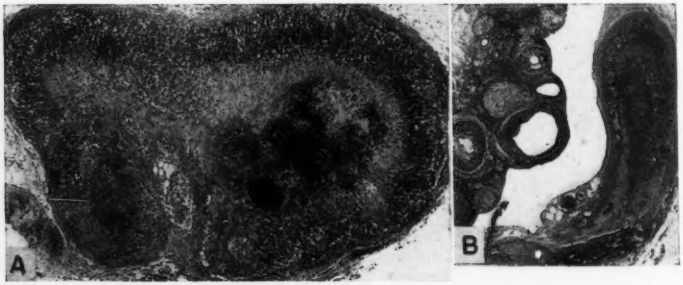

Fig. 2. *A,* Graft of adrenal capsule 2 weeks after both capsules had been grafted to ovaries. x30.
B, Unilateral graft of capsule of the adrenal gland 4 weeks after being grafted to ovary. x17.

peripherally, and in 48 hours the entire gland, except for the capsule and a narrow zone of cortical tissue, had been destroyed (fig. 1*A*). During this time the fibrous attachment to the ovary was established, and blood capillaries extended from the ovary into the adrenal capsule of the graft. Seventy-two hours after grafting (fig. 1*B*), blood capillaries had penetrated throughout the capsule, and a wide band of polymorphonuclear leukocytes, lymphocytes and red blood cells had entered the necrotic zone of the graft. Macrophages or histiocytes had likewise entered the field.

Regeneration of cortical tissue began soon after the graft had become vascularized. Centers of proliferation of cortical cells appeared within the capsule and in the narrow rim of the cortex. Proliferation from these centers gave rise to islands of cortical tissue, which rapidly increased in area and finally coalesced to form the irregularly shaped masses of cortical tissue seen at 2 and 3 weeks after grafting (fig. 2*A* and *B*). Growth from the centers of cellular proliferation in the capsule and peripheral zone of cortex extended toward the center of the graft as rapidly as the leukocytes removed the necrotic tissue. By 5 or 6 weeks, a mass of cortical tissue equal to or greater than that

of the grafted gland had been restored (fig. 1*E*). Medullary tissue was not found in any of the regenerated adrenal glands. Differentiation of the cortical mass into the 3 zones of a normal cortex was not apparent. The cells were small and closely packed in the outer zone beneath the capsule, but well-defined spongiocytes with lipoid droplets occurred in the deeper layers. Both glands regenerated to an approximate equal size.

Unilateral autotransplantation of one entire adrenal. When one gland was left in its bed and the other was transplanted to the ovary, degeneration proceeded in exactly the same way, but the extent of regeneration of the graft was greatly reduced. Vascularization of the capsule and polymorphonuclear leukocytic infiltration occurred, and centers of cortical regeneration appeared, but there were only feeble efforts in the direction of restoration. In the absence of active cortical regeneration to fill the region freed of necrotic tissue by leukocytic activity, the graft was greatly reduced in size. Grafts removed at 6 weeks after transplanting contained but a very narrow layer of large, vacuolated cells beneath the capsule on the side nearest the ovary (fig. 1*F*). This figure roughly illustrates the extent of regeneration in any unilaterally grafted gland, although grafts were removed at frequent intervals for 4 months.

Bilateral autotransplantation of the capsules. When both capsules of the glands were grafted to the ovaries, regeneration of the grafts was as extensive as that which followed bilateral transplantation of the entire gland. As soon as the collapsed capsules had become adherent to the ovaries, they rapidly filled with red blood cells, leukocytes and fibrin. Regeneration likewise began in small foci of the capsule and in the narrow zone of cortex attached to it, as in the grafted whole gland. These centers of regeneration increased in size, and in 5 to 6 weeks a cortical mass, equal to the mass of a normal gland, was restored. At 2 weeks the extent of regeneration of the graft from the capsule was as extensive (fig. 2*A*) as that following bilateral transplantation of the entire gland.

Unilateral autotransplantation of the capsule. When only one gland was removed and its capsule sutured to the ovary, there was but little regeneration. The amount of restored cortex (fig. 2*B*) was about equal to that regenerated when one entire adrenal gland was grafted to the ovary and the other was left in place.

Bilateral and unilateral autotransplantation of the enucleated adrenals. All animals grafted with the enucleated portions of the adrenal glands after the capsules had been removed died within 6 weeks of adrenal insufficiency. Degeneration of the grafts had occurred, and there was no subsequent regeneration of the cortex. There was no regeneration from this portion of the gland when one was grafted to the ovary, and the other adrenal gland remained in its normal position.

COMMENT

Survival of an autograft of the adrenal gland depends on at least two factors: a capsule must be present in the graft, and the organism must have

some physiologic need for the biologic principle elaborated by the adrenal gland.

The growth of the normal gland proceeds from the periphery toward the center. New cortical cells arise in the glomerular region, and during their life-cycle migrate gradually through the zona fasiculata and reticularis, where they are ultimately destroyed. Zwemer (11) has shown that new cortical cells arise from the capsule, and it has been demonstrated in this laboratory that cortical cells of the regenerating adrenal gland of the new-born animal likewise take origin from the capsule. Thus, all the data indicate that the capsule is the source of cortical tissue. Since the enucleated portion of the gland (including the medulla and most of the cortex) when transplanted alone degenerated and failed to become restored, it seems likely that the cortical cell is a fully differentiated cell which has lost its power for regeneration. It is not a question of blood supply, for these enucleated glands were as well vascularized as any graft, but their capacity for growth and further differentiation was lost.

Ever since Halsted's (12) work on parathyroid transplantation, the concept has been variously considered that there must be a deficiency of, or a physiologic need for, the principle of a given endocrine gland in order to insure successful grafts of that endocrine gland. The evidence indicates that there is something in the organism regulating the extent of regeneration of a graft, or preventing its survival. Regeneration of an autograft composed of the adrenal gland will not occur in the presence of even a small amount of functioning adrenal cortex. As has been stated above, we secured very small amounts of regenerated cortex in the unilaterally transplanted organs when either the entire gland or just the capsule was grafted. The presence of one functioning gland seemed to inhibit the regeneration of any portion of the other grafted gland. In previous work (1) we could not get adrenal glands of new-born animals to survive in normal animals. Thus, it seems clear that some regulatory mechanism controls regeneration.

Recently Wyman and tum Suden (13) concluded, from a series of studies on adrenal grafts in adrenalectomized and hypophysectomized animals, that survival of a graft depended on the presence of the adrenotropic principle. They expressed the belief that the available pituitary principle was used in the growth and maintenance of the normal glands, and thus grafts of the adrenal in normal animals without available adrenotropic principle failed to survive. They found that when the normal glands were removed from such grafted animals, the adrenotropic principle of the pituitary body became available for use by the grafts, so that regeneration ensued.

The interpretation of Wyman and tum Suden that the output of adrenotropic hormone from the pituitary body is constant was based in part on an observation, by one of us (14), that transplanted adrenal glands will regenerate, even though the animal is receiving maintenance doses of cortin. Although we also regarded the observation as evidence against a factor of 'physiologic need' for cortin influencing regeneration of transplanted adrenal

cortex, this interpretation must be modified to meet more recent experimental results. We have observed, in experiments yet to be reported, that the presence of one intact adrenal gland will effectively suppress regeneration from the capsules of the other gland after enucleation (removal of medulla and most of cortex, the capsule being left attached to its pedicle). Regeneration from this capsule will occur, however, whenever the opposite intact gland is removed. Although administration of minimal maintenance doses of cortin does not tend to suppress regeneration from the enucleated capsule, Ingle and Kendall (15) found that oral administration of large amounts of cortin will suppress the regeneration just as effectively as the presence of one normal gland. In addition, Ingle and Kendall found that oral administration of large amounts of cortin to the normal rat brings about an atrophy of the adrenal cortex, which is as rapid and extensive as the atrophy of the adrenal cortex resulting from hypophysectomy. This atrophy of the cortex is prevented when an adrenotropic fraction from the anterior lobe of the pituitary body is administered simultaneously with the cortin. Our studies fully support the conclusion of Wyman and tum Suden that regeneration of transplanted adrenal cortex is dependent on the adrenotropic activity of the anterior lobe of the pituitary body. Moreover, we believe that the facts of hypertrophy of the adrenal cortex during conditions of stress, suppression of regeneration of the adrenal cortex in the presence of an excess of its own principle, and the production of atrophy of the adrenal cortex by administration of large amounts of cortin can best be explained by the assumption that the adrenotropic activity of the pituitary body is responsive to the 'physiologic requirements' for cortin. The manner in which the adrenotropic activity of the pituitary body is regulated, and the mechanism by which the adrenotropic hormone influences the adrenal cortex are still unknown.

SUMMARY

Autografts, made from the entire adrenal gland, degenerate except for the capsule and a narrow rim of zona glomerulosa. When both adrenal glands were grafted, entire, regeneration began on the third day, and centers of proliferation occurred in the capsule and in the viable cortical cells. A completely restored cortical mass, equal to or greater than the size of the grafted gland, appeared within 5 or 6 weeks after both adrenal glands, entire, had been grafted. Cortical regeneration in a unilaterally grafted (entire) adrenal gland is very slight when the opposite gland is left intact. Cortical regeneration from bilaterally grafted capsules is as great as that from bilaterally grafted glands. Unilaterally grafted capsules show regeneration of only slight amounts of cortical tissue. There is no cortical regeneration from the enucleated portion of the gland, either when bilaterally or unilaterally grafted. The capsule is essential to regeneration of the gland. Regeneration of a transplanted gland will not occur in the presence of another functional adrenal gland. There is some regulatory mechanism which probably includes the adrenotropic principle of the pituitary body, and which controls the regeneration of any graft of adrenal cortical tissue.

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THE WORK PERFORMANCE OF UNTREATED HYPOPHYSECTOMIZED RATS

DWIGHT J. INGLE
From the Division of Experimental Medicine, The Mayo Foundation
ROCHESTER, MINNESOTA

This report is concerned with the capacity of the gastrocnemius muscle of hypophysectomized rats to sustain work output when subjected to faradic stimulation. At varying periods of time subsequent to operation, the capacity for work was determined for: *a)*, totally hypophysectomized animals; *b)*, animals of which the anterior lobe of the hypophysis had been removed and the posterior lobe left intact; and *c)*, animals of which half of the anterior lobe of the hypophysis had been removed and the posterior lobe left intact.

METHODS

Male rats of the Wistar strain, which were uniform as to age and which weighed between 180 and 190 gm., were used in these experiments. The pituitary body was exposed with a No. 9 dental bone bur, by the usual ventral, parapharyngeal approach. In the operations in which the posterior lobe was left intact, the anterior lobe was divided into two halves before removal. The tissue was removed through a pipet by suction, which was controlled by a foot pedal. Those animals, which had either hemorrhage or dyspnea as a result of the operation, and those concerning which there was a question regarding completeness of the operation, were immediately discarded.

In the work tests (1), the animals were anesthetized with phenobarbital sodium. The gastrocnemius muscle was loaded with 100 gm. and forced to contract 3 times per second to direct faradic stimulation, effected by means of silver needle electrodes. Each stimulus consisted of both make and break shocks, and was of optimal intensity. A maximum of 8 animals could be used simultaneously. The electrodes to each were connected in series, and the same shock stimulated all of the animals. The summation of the heights of all muscular contractions was registered automatically. The animals were enclosed in a cabinet with the temperature constant at 28°C. The initial rate of work per minute was noted, and the animals were observed until the stimulated muscle lost responsiveness, or until a maximum of 120 hours had passed. Those animals working for longer than 24 hours were administered 5 cc. of distilled water subcutaneously, at intervals of 12 hours.

EXPERIMENTS AND RESULTS

In the first experiment, the work performance of 10 rats which had not been subjected to operation was studied. Eight animals of this group worked continuously for 120 hours, at which time experimentation was discontinued.

Two animals of this group lost muscular responsiveness after 96 hours of continuous work.

In the second experiment, the work performance of 90 completely hypophysectomized rats was studied. Ten of these animals were used in the work test immediately after hypophysectomy. The remaining animals were studied in groups of 10, after periods of 1, 2, 3, 4, 5, 6, 7, and 60 days' delay between the operation and the beginning of experimentation. Accurate records of the time for 'fatigue' are not available, but no animal of this series was

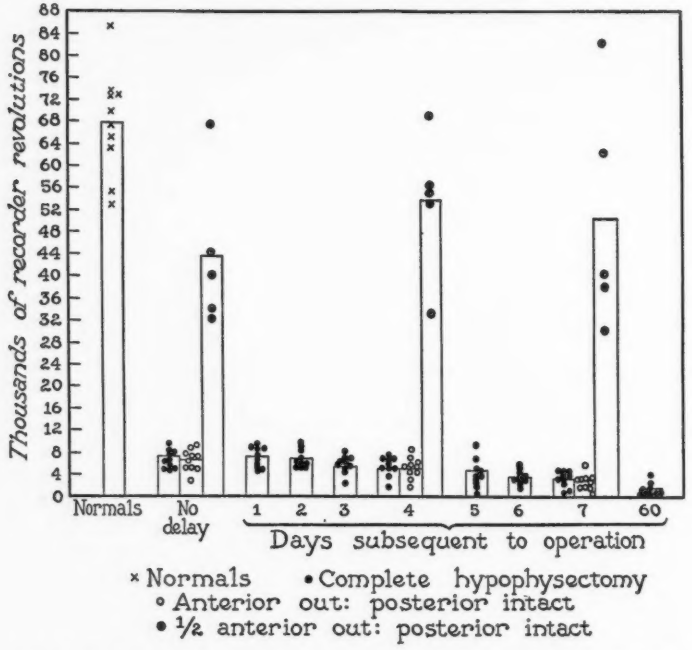

Fig. 1. Averages and individual values for total work performed by hypophysectomized rats.

able to work for as long as 24 hours. The total amount of work performed by each of these animals was very small, and showed a tendency to decrease as the period of delay was increased. The initial rate of work for those animals studied immediately after operation compared favorably with normal standards, but it tended to be reduced as the period of delay was increased.

In the third experiment, a study was made of the work performance of 30 rats, of which the anterior lobe of the pituitary body had been removed but the posterior lobe left intact. Ten of these animals were used in the work test immediately after operation. The remaining animals were studied in groups of 10, after periods of 4 and 7 days' delay. The work performance of these animals was approximately equal to that of completely hypophysectomized animals after identical periods of delay.

In the fourth experiment, the work performance of 15 animals was studied after half the anterior lobe of the pituitary body had been removed. Five of these animals were used in the work test immediately after operation. The remaining animals were studied in groups of 5, after periods of 4 and 7 days' delay. The work performance was far superior to that of animals in which the anterior lobe of the pituitary body had been completely removed. The time for work ranged between 48 and 120 hours, and 7 animals of the group of 15 were sustaining muscular contractions at the end of the 120-hour period. Comparisons of the work totals with those of normal animals showed that there was some overlapping in performance, although the averages of the

TABLE 1. CHANGES IN BODY WEIGHT AND THE INITIAL RATES OF WORK[1] AFTER HYPOPHYSECTOMY

Pituitary body	Rats	Days between operation and experiment	Change in wt., gm.		Initial rate of work	
			Average	Range	Average	Range
Normal	10	0	—	—	16.5	13–20
Completely removed	10	0	—	—	18.0	15–20
	10	1	−33.6	−25 −42	13.5	10–17
	10	2	−26.3	−21 −34	14.3	12–16
	10	3	−30.5	−27 −35	13.2	10–15
	10	4	−27.1	−20 −34	11.0	9–14
	10	5	−35.9	−25 −50	9.1	2–14
	10	6	−32.5	−20 −45	7.7	7– 9
	10	7	−39.9	−30 −56	9.0	7–11
	10	60	−48.6	−41 −62	6.4	4– 8
Anterior lobe removed	10	0	—	—	16.4	12–18
	10	4	−30.5	−20 −37	12.4	9–17
	10	7	−38.3	−30 −53	9.6	7–13
Half of anterior lobe removed	5	0	—	—	18.0	16–24
	5	4	− 6.2	0 −13	16.8	13–20
	5	7	+ 3.0	−19 +18	14.6	11–18

[1] Work is expressed in terms of recorder revolutions. Each recorder revolution is equal to approximately 400 gm.-cm. of work.

work totals of rats, of which half of the anterior pituitary lobe had been removed, remained below normal.

The records for total work are summarized graphically in figure 1, and the data on changes in weight and the initial rates of work are presented in table 1.

At necropsy it was found that the object of each operation had been fulfilled, in so far as could be determined by macroscopic examination.

COMMENT

The general finding that the capacity of hypophysectomized rats to sustain work output is greatly decreased below normal standard could be readily anticipated. It is of interest that the capacity to work remains normal for a few hours after operation, and is then rapidly decreased. This early loss of capacity to continue work appears before any of the other symptoms of pituitary insufficiency, and it is reasonable to attribute it to the loss of the anterior lobe of the pituitary rather than to the operative procedure itself. The work performance of rats, in which only half of the anterior lobe had been

removed, was far superior to that of animals in which the anterior lobe had been completely removed, although the amount of trauma must have been approximately equal in each operation.

The presence of the posterior lobe of the pituitary body affords no protection against the effects of removing the anterior lobe in so far as capacity to work is concerned. The criticism that the functional activity of the posterior lobe may be lost because of circulatory disturbances, owing to removal of the anterior lobe, cannot be satisfactorily answered. But it was noted that the animals of which the posterior lobe had been left intact did not manifest the polydipsia which, for a few days subsequent to operation, invariably affected the animals from which the pituitary body had been completely removed. Conclusions regarding the relationship of the posterior lobe of the pituitary body to the capacity for work cannot satisfactorily be drawn until experiments have been conducted on animals in which the posterior lobe has been removed and the anterior lobe left intact.

In reporting studies on the work performance of adrenalectomized rats, Hales, Haslerud and Ingle (2) pointed out that the loss of capacity for work should not be regarded as a specific 'fatigue' of the stimulated muscle. This caution applies equally well to the present study. The stimulated muscle loses its capacity to contract because of the development of a general state of shock. The capacity for response is also lost in muscles not previously stimulated. The physiologic changes which lead to this shock-like state are not known. Death of the animal occurred within a few hours after the muscle had ceased to respond. The height of muscular contraction may be regarded as providing an indication of the approach to, or departure from, a state of normality, and in this regard it should be of interest to study the influence of various hormones of the anterior lobe on the capacity for work of the hypophysectomized rat.

SUMMARY

When the gastrocnemius muscle of a rat is stimulated immediately following hypophysectomy, the rate of work is normal during the first few hours, but then rapidly decreases to a point at which muscular responsiveness is completely lost. As the interval of time between the operation and the beginning of the work test is increased, the capacity of the muscle to sustain work is decreased. The presence of the posterior lobe of the pituitary body does not protect the animal against the deficiencies in capacity to work, which develop when the anterior lobe is completely removed. If half of the anterior lobe is left intact, the work performance of the rat is carried out at a nearly normal level.

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SOME EFFECTS OF ADRENALECTOMY IN FOWLS[1]

EARL H. HERRICK AND OLAF TORSTVEIT
Kansas State Agricultural Experiment Station, Department of Zoology
MANHATTAN, KANSAS

That the adrenal cortex has a functional association with the gonads was first suggested a number of years ago, and relatively recent work has added much strength to this view. Feeding adrenal cortex or entire adrenal glands to experimental animals has produced maturity earlier than usual or stimulation of the gonads, according to the work of Chidester, Eaton and Thompson (1), McKinley and Fisher (2), Vicent (3), Hewer (4), and several others. The work of Casida and Hellbaum (5), Corey and Britton (6, 7, and 8), and Grollman and Howard (9), has demonstrated that administration of cortical extracts has likewise stimulated early development of the gonads, or increased their activity. The removal of the adrenal glands, on the other hand, has been described by Britton and Kline (10), Carr (11), Corey and Britton (8), Martin (12), Wyman (13) and Freed, Brownfield and Evans (14) as resulting in lowered fertility, interruption or complete suppression of the estrous cycle, or degeneration of the gonads. Rats were used largely in these various investigations.

Adrenalectomy in birds has apparently received very little attention, for a search of the literature has revealed but one report of this operation. Gourfein (15) in 1896 removed the adrenal glands from pigeons, with the conclusion that death followed in from 5 to 24 hours. In this short time it was impossible to observe any effects the lack of adrenals might have on other glands. Probably an important reason that so little work has been done is the difficulty of removing the glands in birds. The adrenals are so vascular and located so close to the aorta, and also the vena cava, that hemorrhage is a great problem to overcome. The close proximity to the lungs and kidneys adds to the difficulty. We believe the work reported in this paper is the first successful adrenalectomy in birds in which observations were made over a period of time.

METHODS AND RESULTS

The prominence of the headdress and its responsiveness to conditions in the gonads make the domestic chicken an usually suitable animal for the study of the effects of adrenalectomy on primary and secondary sexual characteristics. First attempts proved it difficult if not impossible to remove the glands by means suitable to other animals. The slightest attempt to break an adrenal loose from its attachment resulted in a fatal hemorrhage, or one so severe that the operation could not be continued. Ligation of the blood vessels run-

[1] Contribution No. 184, Department of Zoology.

ning to the adrenals seemed to be out of the question, and because of this difficulty cautery was resorted to as a means of destroying the adrenal tissue.

The cauterizing instrument, made in our laboratory but resembling those used in some medical practice, consisted of flexible copper wires running to a short loop of nichrome wire. It was important to control the voltage of the electric current so that the cautery tip would show a dull red glow. If the tip was too hot, it cut too much like a knife and caused hemorrhage and, if not hot enough, progress could not be made because of insufficient destruction of tissue. By careful manipulation the glands could be destroyed completely, even though the tissue is closely attached to, and partly surrounds, major blood vessels. The destruction of gland tissue by means of cautery is the only method of adrenalectomy we have been able to use, and even then numerous birds were lost from hemorrhage.

Birds were prepared for cauterization of the glands by withholding food for 24 hours. They were anesthetised by using nembutal intravenously. The body was opened much as in caponizing, between the last two ribs as far dorsally as possible. A screw-controlled retractor was used to spread the ribs apart and facilitate working. After the gland was destroyed, the ribs were pushed back as nearly as possible to their original positions and the opening bridged across with several stitches of silk, linen, or even cotton thread. The skin was drawn together with fine suture thread. The wounds usually healed in a few days, and no further attention was needed. Antiseptic solutions were used on the instruments and the operator's hands, but rigid aseptic conditions were not maintained. No infections occurred. Only one gland was removed at a time, and one week or longer elapsed between operations. Before the incision was closed during the second operation, one gonad was measured for length and width. The comb was also measured.

Immediately following the removal of the second adrenal gland, 1 cc. of cortical extract, eschatin, was injected intramuscularly. This was repeated during the next 2 or 3 days, 1 cc. daily, divided into two doses. Drinking water containing 16 gm. of sodium chloride per liter was available to the birds at all times. Adult chickens drank from 1 to 2 liters of this salt water every day. After eschatin was discontinued, the injection of normal saline was begun giving 12 to 15 cc. intramuscularly every day. However, this amount was divided into 2 or 3 doses. Adrenalectomized birds thus treated lived from a few days to as long as 82 days. Mature birds usually lost a few gm. in weight after the operation, but later regained their normal weight. Young birds continued to grow after the removal of the adrenals, but not so rapidly as their controls.

Since different breeds of chickens mature at different ages and their secondary sexual characteristics are expressed somewhat differently, a single breed (single comb white Leghorn) was used. Birds from 2 months to nearly 1 year in age were used, but the greater number were 5 to 6 months old, at which age the testes had undergone enlargement. Only the work on male birds is being reported here, since adrenalectomy in females necessitates slightly different operative procedure and only a little work has been done with them.

If cortical hormone or sodium salts are not administered, chickens die from 6 to 15 hours after the second adrenal is removed. The typical symptoms of cortical insufficiency in mammals did not appear in adrenalectomized chickens. The birds seemed quite normal until shortly before their death, when muscle weakness was the principal symptom. Adrenalectomized birds maintained on cortical extract and salt were normal in general appearance, except that from 2 to 4 days after the second adrenal was removed the comb began to be somewhat paler in color and less turgid. Changes in the comb continued until marked reduction in size was evident (fig. 1). In the course of a few days to a few weeks, the adrenalectomized birds had the appearance of typical capons. The combs were pale in color and greatly reduced in size,

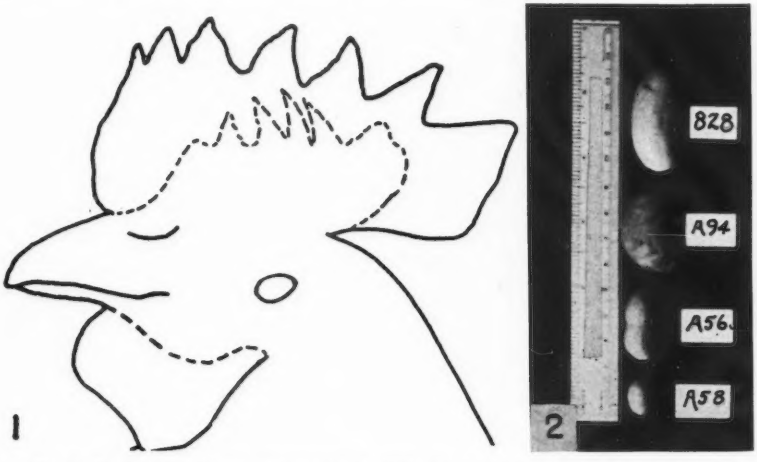

Fig. 1. Drawing traced from photographs of *A300*, showing original size of comb and size 82 days after adrenalectomy.
Fig. 2. Photograph of testes of control and adrenalectomized birds *828* and *A94* normal; *A56* 14 days and *A58* 17 days after adrenalectomy.

the sickle feathers of the tail and the hackle feathers of the neck were indistinguishable from those of a capon. In immature birds the leg bones grew to a greater length than their controls.

The change in the testes was even more striking. As early as 4 days after complete adrenalectomy the testes were noticeably smaller. Their reduction continued until they were only a fraction of their original size (fig. 2). Histological study of the testes from experimental birds revealed marked degeneration as well as reduction in size. The tubules were reduced in size, and the Sertoli cells were separated from the interstitial cells. The walls of the tubules were so broken down that no lumina were distinguishable (fig. 3 and 4).

Measurements of the combs were carefully made every week. The testes were measured at the time of adrenalectomy and again at the time of death or whenever the bird was sacrificed. In a few cases, birds were opened and the testes measured between the operation and postmortem examination. More than 40 chickens were used in this series of experiments.

Actual results from typical adrenalectomized chickens follow. *Bird A56:* 8½ months old at operation; survival period 14 days; change in comb noted 2 days after operation; original size of testes 25 mm. long, 17 mm. wide; size of testes at death 15 mm. long, 13 mm. wide; original size of comb 111 mm. long, 75 mm. high; size of comb at death 97 mm. long, 55 mm. high. *Bird A38:* 3⅓ months old at operation; survival period 35 days; change in comb after 4 days; original size of testes 13 mm. long, 3 mm. wide; size of testes at death 8 mm. long, 3 mm. wide; original size of comb 63 mm. long, 30 mm. high; size of comb at death 45 mm. long, 20 mm. high. In bird *A232,* the testes showed a decrease in length from 26 mm. to 11 mm. in 53 days. The testes of bird *A60* underwent a decrease from 34 to 25 mm. in length in

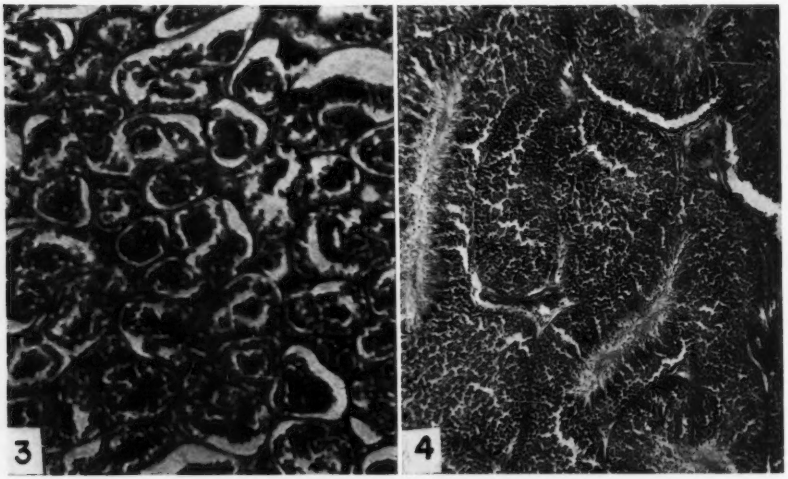

Fig. 3. Cross section of testis of bird 17 days after adrenalectomy.
Fig. 4. Cross section of testis of normal bird, same age and magnification as in figure 3.

32 days. The comb of bird *A300* decreased in length from 81 to 54 mm. in 82 days, and the height of this comb decreased from 54 to 33 mm. in the same length of time.

In no case of complete adrenalectomy did the testes and the comb fail to undergo reduction in size. It is believed these results are not due to the effects of the operation other than the destruction of adrenal tissue, for if a small quantity of adrenal tissue is left intact, the changes in the comb and testes do not occur. The operative procedure in these cases was the same as if the whole gland were removed. Since the heating element of the cauterizing instrument was so small, there was no appreciable injury to tissues adjacent to the adrenal glands.

SUMMARY

We believe this is the first time adrenalectomy has been accomplished in birds maintained long enough to study the effects over a period of time. The glands were destroyed by cauterizing. Adrenalectomized chickens have been

maintained for more than 80 days by giving a few injections of cortical hormone and continued treatment with sodium chloride.

Birds receiving no special treatment die in 6 to 15 hours after complete adrenalectomy. The combs of adrenalectomized chickens become greatly reduced in size. Changes appear as early as the second day after complete adrenalectomy. The testes of adrenalectomized chickens also become smaller. The histological structure of the testes is altered, indicating marked degeneration, and the walls of the tubules are so broken down that the lumina are obscured.

We believe that the adrenal glands have an important influence in maintaining the testes of chickens. Experiments have not as yet indicated the nature of this influence, or whether it reaches the testes directly or indirectly. Likewise, it has not been determined whether the influence on the comb is due to a lack of adrenal secretion directly, to reduced testicular activity, or to some other indirect effect of adrenalectomy. The changes in the comb begin so soon after adrenalectomy, at which time the testes are still large, that it is questionable whether reduced testicular activity is the entire answer.

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THE FREE AND TOTAL CHOLESTEROL CONTENT OF WHOLE BLOOD AND PLASMA AS RELATED TO EXPERIMENTAL VARIATIONS IN THYROID ACTIVITY

L. H. SCHMIDT AND HETTIE B. HUGHES
From the Christ Hospital Research Institute and the Department of Biochemistry, College of Medicine, University of Cincinnati
CINCINNATI, OHIO

There have been numerous clinical studies of the relation between thyroid activity and blood cholesterol content, the results of which have been widely divergent. The few experimental studies have given similarly conflicting results, hence, further experimental work seems justified in order to define more clearly the relations between thyroid activity and blood cholesterol content, to explain the discrepancies in the existing data, and to obtain a theoretical explanation for the changes in blood cholesterol which do occur.

The present study concerns the variations in whole blood and plasma cholesterol in experimental hypothyroidism and hyperthyroidism in the dog. Both free and total cholesterol have been measured. The theoretical importance of considering the cholesterol partition is obvious, but this has not heretofore been done in an experimental study.

EXPERIMENTAL

The experiments were arranged to show *a)*, the normal fluctuations in the free and total cholesterol content of whole blood and plasma of normal dogs during a period of 5 to 6 months; *b)*, fluctuations in thyroidectomized dogs; *c)*, fluctuations in normal dogs made hyperthyroid by ingestion of thyroxine; and *d)*, fluctuations in thyroidectomized dogs after ingestion of thyroxine. For these purposes 9 dogs were treated in the following manner. Two dogs, No. *1* and *2*, served as untreated normal controls during the entire experiment. Dogs *3* and *4* were studied as normal controls for 9 weeks, then thyroidectomized and studied as 'thyroidectomy' controls for the remaining 18 weeks. A third group of 5 dogs, *5* to *9*, were studied as normal controls for 4 weeks, then fed varying amounts of thyroxine (Oral Thyroxine, Squibb) for the next 5 weeks. At the end of this period, these dogs were thyroidectomized and studied as thyroidectomy controls for 5 weeks, after which thyroxine was fed for another 4 weeks; the remaining 9 weeks served as a recovery period from thyroxine treatment. The effects of these procedures on body weight are shown in table 1.

These dogs were all mongrels and, with the exception of dog *2*, had been raised in our laboratory. Dogs *6* and *7* were litter mates 3 years old; dog *1* was 2 years old; dogs *3*, *4*, *5*, *8*, and *9* were litter mates 10 months old. Unless

otherwise indicated, their diet consisted of 400 gm. of salmon bread (1), ½ pint of fresh vegetable soup and 1 pint of milk per day.

In performing the total thyroidectomies, the parathyroids were also removed and transplanted into the sternothyroid muscle. These transplants did not take, and tetany appeared in all dogs within 6 days of the thyroidectomy. In order to relieve this tetany and prevent its recurrence, parathyroid extract (Lilly) was administered daily in such amounts as were necessary to keep

TABLE 1. VARIATIONS IN THE BODY WEIGHT

Date	Normal Control Dogs		Thyroidectomy Control Dogs		Thyroxine—Thyroidectomy—Thyroxine Dogs				
	1	2	3	4	5	6	7	8	9
			No Treatment		No Treatment				
	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.
11/12/36	12.5		10.5	12.6	15.0	13.3	15.9	7.4	8.2
12/10/36	11.8		10.6	12.8	14.5	12.8	15.7	7.6	8.8
			No Treatment		Dogs *5* and *6:* 2 mg. thyroxine daily, from 12/10/36 to 1/12/37. Dogs *7*, *8* and *9:* 0.8 mg. thyroxine daily, from 12/10/36 to 1/13/37.				
12/24/36	11.9		10.9	12.9	15.2	12.6	15.1	7.6	9.2
1/14/37	11.9	27.5	10.6	13.2	15.3	12.0	14.4	6.8	9.2
			Thyroidectomies 1/18 and 1/21/37		Thyroidectomies 1/15, 16, 20, and 22/37				
2/ 4/37	11.4		12.4	13.8	16.8		16.6	8.6	9.6
2/18/37	11.7	—	12.8	14.8	17.9	—	17.4	10.5	10.5
			No Treatment		All dogs: 0.8 mg. thyroxine daily, from 2/18/37 to 3/3/37 and 2 mg. thyroxine daily, from 3/4/37 to 3/18/37				
3/ 4/37	12.1	—	13.3	14.8	15.9	—	15.8	9.0	9.3
3/18/37	12.1	—	12.9	14.1	14.8	—	15.1	8.6	9.0
			No Treatment		No Treatment				
4/ 1/37	13.0	—	12.9	14.5	13.6	—	16.6	8.8	9.6
5/18/37	12.9	29.0	13.5	14.4	—	—	16.9	8.6	10.0

the serum calcium between 8.5 and 10 mg. per cent. Treatment with parathyroid extract was continued to the end of the experiment.

All of the thyroidectomized dogs were autopsied, particular attention being paid to the site of thyroidectomy. One dog (No. *3*) regenerated a small piece of thyroid tissue, measuring 2 x 5 mm., which was proliferating actively at the time of death.

Blood samples were obtained at the intervals indicated in the tables. After a 20-hour fast, approximately 15 cc. of blood was drawn from the left ventricle and heparinized. A portion of this blood was placed in a calibrated centrifuge tube and centrifuged for 1 hour at 2000 R.P.M. in order to obtain the cell and plasma ratios (table 2). The error of these determinations was ± 2 volume per cent.

Total and free cholesterol determinations were made on alcohol-ether extracts of whole blood and plasma, prepared according to Boyd (2). Total cholesterol was determined colorimetrically by the method of Bloor (3) modified to the extent of carrying out the color reaction in the dark room at 25° C. Free cholesterol was determined oxidatively by the method of Yasuda (4). The experimental errors were ±2 per cent for the free cholesterol and ±4 per cent for the total. The total cholesterol content of the corpuscles was calculated from the whole blood and plasma cholesterol values and the cell

TABLE 2. VARIATIONS IN PLASMA VOLUME[1]

Date	Normal Control Dogs		Thyroidectomy Control Dogs		Thyroxine—Thyroidectomy—Thyroxine Dogs				
	1	2	3	4	5	6	7	8	9
			No Treatment		No Treatment				
11/12/36	48	—	55	54	52	49	45	52	48
12/10/36	45	—	50	48	51	45	45	51	47
			No Treatment		Dogs *5* and *6:* 2 mg. thyroxine daily, from 12/10/36 to 1/13/37 Dogs *7*, *8* and *9:* 0.8 mg. thyroxine daily, from 12/10/36 to 1/13/37				
12/24/36	44	—	49	50	51	47	43	49	49
1/14/37	45	51	48	47	46	47	44	47	46
			Thyroidectomies 1/18 and 21/37		Thyroidectomies 1/15, 16, 20 and 22/37				
2/ 4/37	43	48	52	53	51	—	49	51	51
2/18/37	41	49	56	54	55	—	50	55	51
			No Treatment		All dogs: 0.8 mg. thyroxine daily, from 2/18/37 to 3/3/37, and 2 mg. thyroxine daily, from 3/4/37 to 3/18/37				
3/ 4/37	42	47	54	50	48	—	51	50	49
3/18/37	40	47	53	50	50	—	52	49	49
			No Treatment		No Treatment				
4/ 1/37	43	44	54	—	53	—	52	51	51
5/18/37	45	47	48	51	—	—	50	56	53

[1] Plasma volume expressed as per cent of whole blood.

and plasma volumes; obviously, these values were subject to all the errors of the other determinations. The free cholesterol content of the corpuscles was also calculated but not recorded; it was of little significance since, due to the accumulation of errors, several of the free cholesterol values exceeded those for total cholesterol.

RESULTS

Fluctuations in normal control dogs. During the entire 6 months of study (tables 3, 4 and 5), the amounts of total cholesterol in the plasma, whole blood and blood corpuscles varied less than 20 per cent from mean values. In

TABLE 3. VARIATIONS IN THE TOTAL CHOLESTEROL AND FREE CHOLESTEROL CONTENT OF PLASMA

Date	Normal Control Dogs						Thyroidectomy Control Dogs						Thyroxine-Thyroidectomy-Thyroxine Dogs														
	1			2			3			4			5			6			7			8			9		
	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]
							No Treatment						No Treatment														
11/12/36	109	22	20				146	41	28	124	31	25	129	26	20	98	25	25	100	22	22	121	34	28	121	28	23
12/10/36	143	27	19				176	40	23	156	34	22	155	31	20	102	24	24	100	17	17	135	31	23	160	35	22
							No Treatment						Dogs *5* and *6:* 2 mg. thyroxine daily, from 12/10/36 to 1/13/37 Dogs *7*, *8* and *9:* 0.8 mg. thyroxine daily, from 12/10/36 to 1/13/37														
12/24/36							145	33	23	134	33	25	145	35	24	80	15	19	99	19	19	132	30	23	143	34	24
1/14/37	127	30	24	138	33	24	126	34	27	130	30	23	124	26	21	94	19	20	93	18	19	107	25	23	131	29	22
							Thyroidectomies 1/18 and 21/37						Thyroidectomies 1/15, 16, 20 and 22/37														
2/ 4/37	114	25	22	130	31	24	220	51	23	223	60	27	223	60	27				220	57	26	226	61	27	263	63	24
2/18/37	151	32	21	144	36	25	311	81	26	256	59	23	345	69	20				284	60	21	293	64	22	280	67	24
							No Treatment						All dogs: 0.8 mg. thyroxine daily, from 2/18/37 to 3/3/37, and 2 mg. daily, from 3/4/37 to 3/18/37														
3/ 4/37	137	33	24	127	31	24	290	73	25	231	53	23	172	40	23				123	31	25	148	36	24	146	29	20
3/18/37	131	30	23	143	33	23	193	44	23	213	49	23	102	22	22				83	22	26	100	20	20	105	21	20
							No Treatment						No Treatment														
4/ 1/37	132	32	24	114	31	27	219	59	27				143	36	25				162	39	24	104	25	24	138	33	24
5/18/37	132	30	23	123	36	29	219	57	26	253	66	26							280	64	23	140	39	28	192	44	23

[1] T = mg. of total cholesterol per 100 cc. of plasma.
[2] F = mg. of free cholesterol per 100 cc. of plasma.
[3] PF = per cent of total plasma cholesterol as free cholesterol.

dog *1*, plasma cholesterol varied between 109 and 151 mg. per 100 cc., that of whole blood between 126 and 156 mg., and that of blood corpuscles between 124 and 162 mg.; smaller variations were found in dog *2*. The variations in total cholesterol content of plasma were within the limits reported by Bloor (5) for his normal dogs.

The absolute amount of free cholesterol in both plasma and whole blood fluctuated to about the same extent as the total cholesterol, but the per cent of free cholesterol was remarkably constant for each dog (tables 3, 4, and 5). In dog *1*, free cholesterol constituted from 19 to 24 per cent of the total plasma cholesterol, and from 58 to 68 per cent of that of whole blood.

Fluctuations in the 'thyroidectomy' control dogs. In the pre-operative control period, the amounts of total and free cholesterol in the plasma, whole blood and corpuscles of dogs *3* and *4* were not essentially different from those of dogs *1* and *2* (tables 3, 4 and 5). The per cent of free cholesterol in the whole blood of dog *3* appeared to be less than that of the other controls. This was not due to a deficiency of free cholesterol in either plasma or corpuscles, but to a high plasma cholesterol content combined with a large plasma volume (table 2).

Thyroidectomy produced a plasma hypercholesterolemia (table 3). In less than 3 weeks after thyroidectomy, both the total and the free cholesterol content of plasma increased significantly, more than 70 per cent. Since the increase in free cholesterol was roughtly proportional to that in total cholesterol, the relative proportions of free cholesterol remained constant.

Thyroidectomy also produced a whole blood hypercholesterolemia (table 4), but the increase in whole blood cholesterol was less than that in the plasma; e.g., in dog *4* whole blood cholesterol increased from 132 to 185 mg. per 100 cc., while plasma cholesterol increased from 132 to 223 mg. The amount of free cholesterol increased, but to a lesser extent than the total cholesterol; thus, the per cent of free cholesterol actually decreased. Since the total cholesterol content of the corpuscles was not affected by thyroidectomy (table 5), the increase in whole blood cholesterol and the decrease in per cent of free cholesterol were merely reflections of the plasma changes.

The plasma hypercholesterolemia reached a peak approximately 5 weeks after thyroidectomy (table 3). At that time, the total cholesterol content of the plasma of dog *3* was 311 mg. per 100 cc., that of dog *4* 256 mg., as compared with pre-operative levels of 126 and 130 mg., respectively. Subsequent analyses made at varying intervals during the next 13 weeks showed that the plasma cholesterol of dog *4* remained at this high level. Such was not the case with dog *3;* by the ninth postoperative week (3/18/37) the plasma cholesterol had dropped to 193 mg., and remained at or near this point during the remainder of the experiment. The presence of the active remnant of thyroid gland probably accounts for this reaction.

Fluctuations in dogs treated with thyroxine before and after thyroidectomy. In the pretreatment control period, the amounts of free cholesterol in the whole blood, plasma and blood corpuscles of dogs *5* to *9* were similar to those of dogs *1* to *4* (tables 3, 4 and 5) with the exception that the amounts of

TABLE 4. VARIATIONS IN THE TOTAL CHOLESTEROL AND FREE CHOLESTEROL CONTENT OF WHOLE BLOOD

Date	Normal Control Dogs						Thyroidectomy Control Dogs						Thyroxine-Thyroidectomy-Thyroxine Dogs														
	1			2			3			4			5			6			7			8			9		
	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]	T[1]	F[2]	PF[3]
							No Treatment						No Treatment														
11/12/36	137	79	58				157	83	53	142	88	62	135	74	55	129	80	62	126	77	61	125	82	66	148	87	59
12/10/36	145	90	62				161	89	55	147	90	61	149	83	56	128	78	61	121	81	67	140	87	62	148	93	63
							No Treatment						Dogs 5 and 6: 2 mg. thyroxine daily, from 12/10/36 to 1/13/37 Dogs 7, 8 and 9: 0.8 mg. thyroxine daily, from 12/10/36 to 1/13/37														
12/24/36	134	80	60				134	75	56	125	79	63	132	79	60	109	70	64	121	77	64	133	85	64	128	81	63
1/14/37	129	82	64	142	99	70	126	73	58	132	82	62	122	73	60	111	74	67	114	79	69	118	78	66	140	88	63
							Thyroidectomies 1/18 and 21/37						Thyroidectomies 1/15, 26, 20 and 22/37														
2/ 4/37	133	82	62	141	96	68	197	96	49	185	92	50	192	90	47				184	96	52	188	90	48	221	102	46
2/18/37	156	90	58	153	98	64	241	106	44	207	108	52	268	105	39				220	101	46	229	108	47	230	117	51
							No Treatment						All dogs: 0.8 mg. thyroxine daily, from 2/18/37 to 3/3/37, and 2 mg. daily, from 3/4/37 to 3/18/37														
3/ 4/37	129	85	66	141	96	68	220	110	50	178	94	53	142	75	53				127	74	58	142	94	66	135	82	61
3/18/37	140	95	68	143	97	68	169	95	56	173	97	56	123	79	64				111	79	71	118	77	65	119	86	72
							No Treatment						No Treatment														
4/ 1/37	141	90	64	147	101	69	192	92	48				140	85	61				155	79	51	125	80	64	146	82	56
5/18/37	140	90	64	147	98	67	192	90	47	202	107	53							222	98	44	147	76	52	179	89	50

[1] T = mg. of free cholesterol per 100 cc. of whole blood.
[2] F = mg. of free cholesterol per 100 cc. of whole blood.
[3] PF = per cent of whole blood cholesterol as free cholesterol.

total cholesterol in the plasma of dogs 6 and 7 were smaller than those of any other dogs.

Ingestion of 0.8 to 2 mg. of thyroxine, for a period of 5 weeks, had no significant effect on either the free or the total cholesterol content of plasma or whole blood (tables 3 and 4). Basal metabolic rates were not determined, but according to Kunde (6) these amounts of thyroxine were sufficient to increase the basal metabolism 50 to 60 per cent. Physical activity, excitability

TABLE 5. VARIATIONS IN THE TOTAL CHOLESTEROL CONTENT OF THE BLOOD CORPUSCLES

Date	Mg. of Total Cholesterol per 100 cc. of Blood Corpuscles								
	Normal Control Dogs		Thyroidectomy Control Dogs		Thyroxine-Thyroidectomy-Thyroxine Dogs				
	1	2	3	4	5	6	7	8	9
			No Treatment		No Treatment				
11/12/36	162	—	171	163	142	156	147	129	173
12/10/36	147	—	146	139	143	149	138	145	138
			No Treatment		Dogs *5* and *6:* 2 mg. thyroxine daily, from 12/10/36 to 1/13/37 Dogs *7*, *8* and *9:* 0.8 mg. thyroxine daily, from 12/10/36 to 1/13/37				
12/24/36	—	—	124	116	119	134	137	133	114
1/14/37	131	114	127	134	120	127	130	128	148
			Thyroidectomies 1/18 and 21/37		Thyroidectomies 1/15, 16, 20 and 22/37				
2/ 4/37	147	152	173	144	161	—	149	147	179
2/18/37	159	162	152	148	177	—	156	151	178
			No Treatment		All dogs: 0.8 mg. thyroxine daily, from 2/18/37 to 3/3/37, and 2 mg. thyroxine daily, from 3/4/37 to 3/18/37				
3/ 4/37	124	153	139	126	116	—	131	136	116
3/18/37	147	142	143	132	144	—	142	135	133
			No Treatment		No Treatment				
4/ 1/37	147	173	161	—	136	—	148	147	155
5/18/37	147	170	167	149	—	—	164	157	164

and desire for food were certainly increased, but the body weight was not affected (table 1). This may have been due to the increase in salmon bread ration from 400 to 600 gm. per day. In any event, the treatment was severe enough to lead to the death of dog 6 with symptoms of thyrotoxicosis (increased respiration, extreme nervousness and diarrhea).

Thyroidectomy performed immediately after this treatment led to a plasma and whole blood hypercholesterolemia, which reached a peak in 5 weeks and appeared to be identical in all respects to that described for the 'thyroidectomy' controls. Four days after thyroidectomy, the salmon bread rations were reduced to 400 gm. daily; on this intake, the dogs gained weight just as did the 'thyroidectomy' controls (table 1).

When these thyroidectomized dogs ingested 0.8 mg. of thyroxine daily

for 2 weeks, both plasma and whole blood cholesterol contents were reduced so that the total cholesterol had returned to or approached the levels of the control period (tables 3 and 4). The amount of free cholesterol in plasma decreased, but the per cent remained unchanged. In whole blood, the absolute amount of free cholesterol decreased but the per cent increased. There was no significant variation in the cholesterol of the blood corpuscles (table 5).

Continued administration of larger amounts of thyroxine intensified the reactions just noted. After ingestion of 2 mg. of thyroxine daily for 2 weeks, the total cholesterol content of the plasma was 7 to 22 mg. lower than the lowest amounts previously found (table 3). The free cholesterol content also decreased, but the per cent of free cholesterol was unchanged. The total cholesterol content of whole blood was slightly lower than that of the preceding series, but none of the values was significantly less than those obtained prior to thyroidectomy (table 4). The per cent of free cholesterol in whole blood was greater than that of any other series; this was due to the small amount of esterified cholesterol contributed by the plasma. The cholesterol content of the corpuscles remained unchanged (table 5).

In contrast to the normal dogs which had been given thyroxine, the thyroidectomized dogs lost considerable weight following such treatment (table 1). Dogs so treated had a tremendous increase in physical activity, but no apparent increase in appetite.

When thyroxine treatments were discontinued, the plasma hypercholesteremia returned, but this was a very slow process as compared with its original appearance. The first evidence of an increase in plasma cholesterol was obtained in dogs 5, 7 and 9 three weeks after the last thyroxine feeding; it was 9 weeks, however, before any of the dogs had a plasma cholesterol content equal to that of the pretreatment period (table 3).

DISCUSSION

Experimental hyperthyroidism of the severity produced in our dogs was not associated with a ~~hyper~~cholesterolemia. This is in accord with the observations of Simonds and Helpler (7), but does not agree with those of Blinoff (8) and Parhon and Derevici (9), who concluded that ingestion of thyroid gland by normal dogs led to a hypocholesterolemia. This apparent discrepancy is due to the fact that the changes in cholesterol ascribed by Blinoff and Parhon to thyroid stimulation fall within the limits of normal variation for our dogs.

Epstein and Lande (10), Mason, Hunt and Hurxthal (11) and Hurxthal (12) found a hypocholesterolemia in cases of exophthalmic goiter, and concluded that in this disorder the blood cholesterol varies inversely with the B.M.R. Our experimental results, as well as numerous clinical observations (13 to 16), make it seem improbable that there is a definite relation between thyroid activity and blood cholesterol in either experimental or clinical hyperthyroidism. It appears more probable that, when a hypocholesterolemia is found in clinical hyperthyroidism, it is due to prolonged malnutrition (17) or to the so-called 'toxic' condition rather than to a specific effect of the thyroid on sterol metabolism.

Experimental hypothyroidism in the dog is associated with a hyper-

cholesterolemia just as is clinical myxedema. The ingestion of thyroxine abolishes this hypercholesterolemia just as it abolishes that of clinical hypothyroidism (13, 18). It is readily apparent that these changes in plasma cholesterol are too large to be entirely due to changes in plasma volume. The observations of Thompson (19) suggested that a significant portion of these variations might be due to hemoconcentration and hemodilution, but such was not the case in our experiments (table 2).

At the present time there is no satisfactory explanation for the hypercholesterolemia associated with hypothyroidism. The increases in blood cholesterol following thyroidectomy, and the subsequent decreases following thyroxine treatment were confined entirely to the plasma, agreeing with the clinical observations of Gardner and Gainsborough (13) and Boyd (20). This indicates the improbability of a general increase in tissue cholesterol. The increase in plasma cholesterol may be due to disturbances in the rate at which this substance is synthesized, destroyed or excreted, or it may merely reflect the rate at which transported fatty acids are oxidized or stored. But whereas the latter explanation might account for the increase in esterified cholesterol, it does not explain the simultaneous increase in free cholesterol.

SUMMARY

Experimental hyperthyroidism in dogs does not produce significant changes in the free and total cholesterol content of either whole blood or plasma. Thyroidectomy produces a marked hypercholesterolemia which reaches a peak 4 to 5 weeks after operation, and appears to be confined entirely to the plasma. This hypercholesterolemia is made up of equal increases in the amounts of free and esterified cholesterol, so that there is no significant change in the per cent of plasma cholesterol existing in the free form. Administration of thyroxine to thyroidectomized dogs abolishes this hypercholesterolemia without disturbing the normal ratio of free to total cholesterol; such treatment does not produce a hypocholesterolemia. These observations are in agreement with the clinical findings in cases of myxedema, and they suggest that the hypocholesterolemia sometimes found in clinical hyperthyroidism is due to factors other than the increased thyroid activity.

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THE PURIFICATION OF THE ANTERIOR PITUITARY GROWTH HORMONE BY FRACTIONATION WITH AMMONIUM SULFATE[1]

HERBERT M. EVANS, NAO UYEI, QUENTIN R. BARTZ AND MIRIAM E. SIMPSON

From the Institute of Experimental Biology
University of California
BERKELEY, CALIFORNIA

Since the discovery in 1921 of a growth-promoting principle in the anterior pituitary by Evans and Long (1), several attempts have been made to purify it. The hormone has always been found associated with proteins and, due to the difficulties inherent in separation of proteins, little progress has been reported in purification.

A brief résumé of the chief methods for preparation of growth-promoting extracts is given below. Evans and Long first used a simple saline extract of the anterior lobes of cattle. Later, Evans and Simpson (2, 3) prepared sodium hydroxide extracts and precipitated some of the contaminating protein by adjustment of the hydrogen ion concentration. Later Evans and co-workers (4, 5) substituted barium hydroxide extraction for sodium hydroxide extraction, and a substantial part of the contaminating proteins was removed by high speed centrifugation. In 1929, Teel (6) fractionated growth-promoting extracts with sodium sulfate, and found the hormone in the globulin fraction. Later van Dyke and Wallen-Lawrence (7) used sodium sulfate fractionation with removal of contaminants at pH 4.75.

More recently, adsorption methods have been used, and the highest potency reported has come from such preparations. Collip, Selye and Thompson (8) have adsorbed on calcium phosphate, whereas Dingemanse and Freud (9) have employed activated carbon (Norit) as an adsorbent, and phenol as an eluent of the hormone. A careful study of both adsorption and salting-out methods has been made in this laboratory, and the latter have proved more satisfactory. It is the purpose of this paper to describe a fractionation with ammonium sulfate, which gives a product of equal potency and somewhat superior yield to that hitherto reported.

STANDARDIZATION OF GROWTH-PROMOTING PREPARATIONS

A). Use of normal female rats. The use of adult female rats for the standardization of growth hormone extracts has already been described (5, 10). In actual practice, a growth hormone preparation was injected into groups of

[1] Aided by generous grants from the Rockefeller Foundation of New York City and the Board of Research of the University of California.

5 or 6 'plateaued'[2] female rats for 20 days (17 injections). A period of 15 days could be used, but any shorter interval has been found unreliable. An apparent growth rate is frequently secured during the first 5 or 10 days which is not further maintained; the growth produced in 20 days bears no satisfactory relation to that produced in 5 or 10 days. The growth hormone was usually given at 3 or more dose levels, the aim being to administer doses which would give growths varying from 30 to 70 gm. A weight increase of less than 40 gm. is not as dependable as an increase of from 40 to 60 gm. We have therefore somewhat arbitrarily taken as a 'unit' of growth hormone the daily dose in dry substance producing a weight increase averaging 40 gm. in 20 days with 6 test rats. A curve may be constructed (5) using the logarithms of the average weight increase as ordinate, and the logarithms of the multiple of the lowest dose as the abscissa. By interpolation, one may obtain the growth to be expected at any particular dose level of any particular preparation; but since the slope of such curves varies with the preparations, it is evident that factors other than the concentration of growth hormone contribute to the result. It is not improbable that thyrotropic hormone is one of these contributing factors.

B). Use of hypophysectomized female rats. As hypophysectomized rats respond by increase in body weight to doses of growth hormone which will not cause dependable weight increases in normal animals, it may be desirable to use them in the standardization of growth hormone preparations (table 1). When hypophysectomized animals are employed for such tests, it is essential that they be rigorously standardized as to strain, sex, age, weight, physiological condition and length of time after hypophysectomy, if comparable results are to be obtained. It is advisable, in order to secure the most dependable data in the injection of very low doses, not to use them repeatedly for growth tests. They are not only less susceptible with continued injection, but the possibility of a gradual constitutional decrease in sensitivity, due to lengthened postoperative life, becomes a factor.

When the unit of growth hormone is based upon the smallest dependable yet clearly discernible growth, the length of the assay period is also of importance in hypophysectomized rats. For practical purposes, the duration of injection should be as short as possible. Reference to table 1 will show that the 5-day assay period is not long enough to recognize a relation between growth and the amount of hormone injected. The gain in weight resulting from different dose levels is less variable after 10 than after 5 daily injections. The gain in a 10-day period is correlated with the amount of hormone injected. The use of a 15 to 20-day injection period may give some additional accuracy to the results, but insufficient to compensate for the disadvantage of the longer test period. Therefore the 10-day injection period has been adopted. The growth hormone unit in hypophysectomized rats may then be defined as that amount which, injected every day intraperitoneally into at least 5 rats, causes an average body weight increase of 10 gm. in 10 days. In this laboratory, female rats

[2] Plateaued females are usually between 5 and 6 months of age, and in our colony weigh 250 to 270 gm.; they may be used until the end of the ninth month. When weighed before treatment at three 5-day intervals, the total spontaneous weight increase must be less than 10 gm.

of the Long-Evans strain 2 to 3 months of age and weighing approximately 150 gm. at hypophysectomy are employed. A period of 20 to 30 days was purposely allowed to elapse between operation and injection.

A study of tables 1 and 2 will show that hypophysectomized rats give a clear-cut response to much smaller doses of growth hormone than do normal adult females. For this reason, and because of the shorter injection period, they may be employed more advantageously where only a small amount of substance is available.

CHEMICAL PROCEDURE

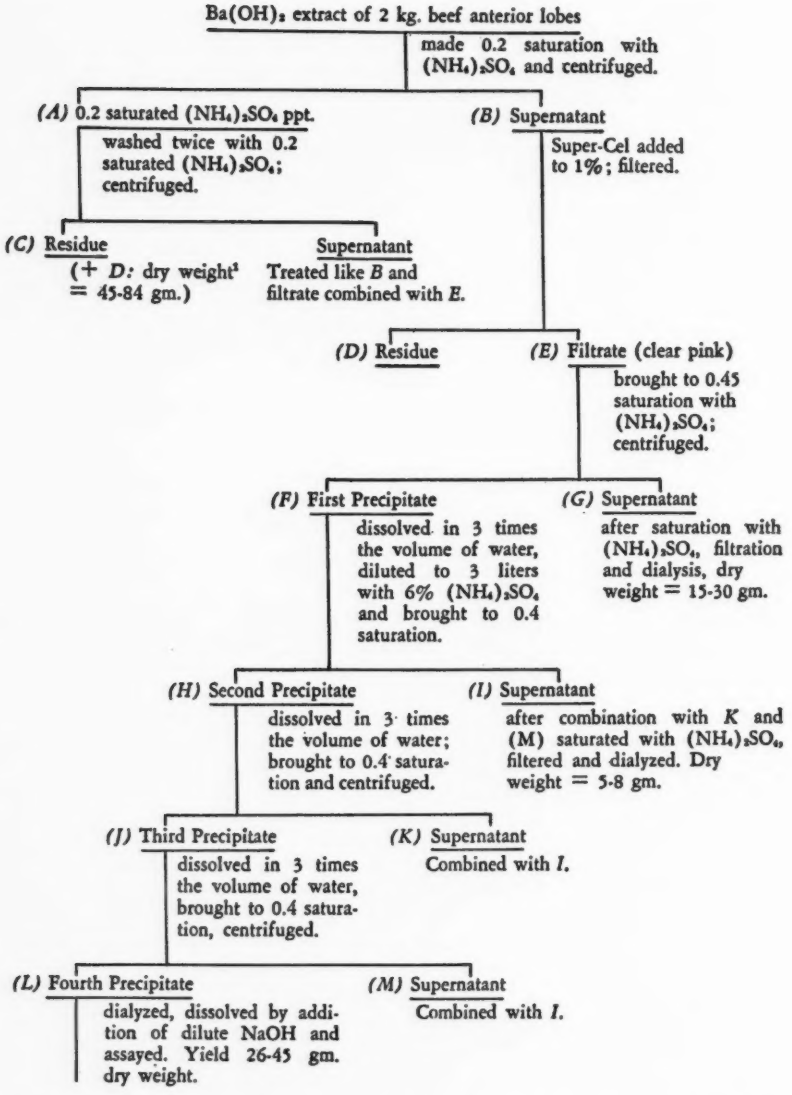
In the first efforts to use ammonium sulfate in purification of the growth hormone, dilute sodium hydroxide extracts of beef anterior lobes were used as the starting material, but it was soon found that a clearer separation of the growth hormone could be obtained when starting with barium hydroxide extracts.

Preparation of the standard alkaline extract. This extract was prepared by a slight modification of the method of Evans, Cornish and Simpson (4, 5). 2000 gm. of frozen beef hypophyses were ground in a Hobart meat grinder. All chemical procedures from this point were conducted at 0° to 3° C. The glands were placed in a large pyrex cylinder and 12 liters of cold distilled water were added. The mixture was stirred until it formed a homogeneous suspension. To the suspension, 2000 to 3000 cc. of 0.2 N barium hydroxide solution was then added slowly with constant stirring; after 15 minutes the pH was determined (glass electrode), which should be between 10.8 and 11.0. The exact amount of barium hydroxide required to bring the mixture to this pH depends on the condition of the original glands, which is influenced by length and conditions of storage. The mixture was allowed to stand over night without further stirring. The extract was then centrifuged repeatedly in a cooled Sharples super-centrifuge. The solution was passed through the centrifuge 4 times, using successively smaller nozzles. This procedure may take 2 days. The solution was then carefully brought to pH 9.0 to 9.5 (glass electrode) by addition of 0.2 N sulfuric acid. After adjustment, 50 gm. of ground sodium sulfate crystals were added with stirring, and the solution was passed through the Sharples supercentrifuge again to remove the barium sulfate formed. A yield of 14.0 to 16.0 liters was usually obtained. The clear cherry-red solution can be stored in frozen state indefinitely. The fractionation of this alkaline extract by ammonium sulfate is shown in the accompanying diagram.

Fractionation of the standard alkaline extract with ammonium sulfate. The alkaline extract (pH 9.0 to 9.5) prepared from 2 kg. of glands was brought to approximately 0.2 saturation by the addition of 121 gm. of ammonium sulfate (C. P., Merck or purified product by J. T. Baker Co.) per liter of extract. The preparation was allowed to stand overnight. As much as possible of the supernatant fluid was then decanted, and the residual liquid containing the precipitate was passed through a chilled Sharples supercentrifuge. This 0.2 saturated ammonium sulfate precipitate *A*[3] consists mainly of proteins which are virtually inactive as regards the promotion of growth. In

[3] The letters in italics refer to the fractions outlined in the diagram.

Schematic Outline of the Method


Ba(OH)₂ extract of 2 kg. beef anterior lobes
made 0.2 saturation with (NH₄)₂SO₄ and centrifuged.
(A) 0.2 saturated (NH₄)₂SO₄ ppt.
washed twice with 0.2 saturated (NH₄)₂SO₄; centrifuged.
(B) Supernatant
Super-Cel added to 1%; filtered.
(C) Residue
(+ D: dry weight[1] = 45-84 gm.)
Supernatant
Treated like B and filtrate combined with E.
(D) Residue
(E) Filtrate (clear pink)
brought to 0.45 saturation with (NH₄)₂SO₄; centrifuged.
(F) First Precipitate
dissolved in 3 times the volume of water, diluted to 3 liters with 6% (NH₄)₂SO₄ and brought to 0.4 saturation.
(G) Supernatant
after saturation with (NH₄)₂SO₄, filtration and dialysis, dry weight = 15-30 gm.
(H) Second Precipitate
dissolved in 3 times the volume of water; brought to 0.4 saturation and centrifuged.
(I) Supernatant
after combination with K and (M) saturated with (NH₄)₂SO₄, filtered and dialyzed. Dry weight = 5-8 gm.
(J) Third Precipitate
dissolved in 3 times the volume of water, brought to 0.4 saturation, centrifuged.
(K) Supernatant
Combined with I.
(L) Fourth Precipitate
dialyzed, dissolved by addition of dilute NaOH and assayed. Yield 26-45 gm. dry weight.
(M) Supernatant
Combined with I.

[1] By dry weight is meant the total solids present after complete removal of ammonium sulfate by dialysis. In the case of *D* the protein is brought into solution by the addition of alkali to pH 9.0 to 9.5 and the Super-Cel is removed by centrifugation. Drying is done to constant weight at 110°C.

order to recover the small amount of growth hormone adsorbed on this precipitate, it was extracted twice with approximately 3 liters of 0.2 (12.1 per cent) saturated ammonium sulfate solution.

The decanted supernatant fluid, the supernatant from the Sharples supercentrifuge and the 0.2 saturated ammonium sulfate extract of the precipitate were treated separately, and Hyflo Super-Cel (Johns-Mansville), 10 gm. per liter, was added to each solution. After homogeneous suspensions were obtained, they were filtered through a Schleicher and Schüll No. 1450½ fluted filter paper. The clear pink filtrates from the 3 solutions were then combined. Treatment of these extracts separately with Super-Cel decreased greatly the time necessary for filtration. The Super-Cel residue *D* on the filters retained a considerable amount of the growth hormone, and it was therefore extracted 3

TABLE 1. COMPARISON OF THE AVERAGE GROWTH[1] OF NORMAL AND HYPOPHYSECTOMIZED FEMALE RATS WHEN INJECTED WITH THE FOURTH AMMONIUM SULFATE PRECIPITATE

Normal Rats					Hypophysectomized Rats								
Daily Dose mg.	Growth, in gm., in days				Daily Dose mg.	Growth, in gm., in days							
						Not Previously Injected				Previously Injected			
	5	10	15	20		5	10	15	20	5	10	15	20
62.00	15	30	50	82	60.00	−4	16	43	70	7	35	64	90
22.00	16	29	50	66									
12.00	17	32	47	62	10.00	4	32	54	76	12	27	38	51
6.00	18	33	45	58									
2.00	14	26	39	52	1.70	17	32	48	62	11	24	37	51
0.67	11	17	23	32									
0.21	9	14	19	15	0.21	13	29	38	46	15	23	28	41
					0.09	10	14	18	20	6	5	7	12
					0.06	12	12	15	17	8	6	3	4
					0.03	5	6	4	5	1	−1	−7	1

[1] Averages based on groups of 5 rats.

times with 0.2 saturated ammonium sulfate solution and the extracts filtered. The latter filtrates were combined with the main filtrate *E*. The combined filtrates were then brought to approximately 0.45 saturation by addition of 151 gm. of ammonium sulfate to each liter. After standing overnight, the precipitate was removed by centrifugation. Practically all of the growth principle was found in the 0.45 saturated ammonium sulfate precipitate *F*. Very little growth hormone was left in the supernatant *G*. When brought to full saturation by addition of solid ammonium sulfate, the precipitate (after dialysis) did not stimulate growth in normal animals in a 20 mg. daily dose. It contained, however, a moderate amount of gonadotropic (chiefly follicle-stimulating) and thyrotropic hormone.

The precipitate containing the growth hormone was washed 3 times by suspension in 0.45 saturated ammonium sulfate solution (277 gm. of ammonium sulfate to a liter of distilled water). All washings were combined with the main supernatant *G*. After washing with 0.45 saturated ammonium sulfate, the precipitates were collected in centrifuge cups, and the approximate volume of the precipitate was measured; 3 times this volume of distilled water

was added to the precipitate and the mixture stirred. Most of the precipitate went into solution. Any undissolved substance was extracted repeatedly with 0.1 (6 per cent) saturated ammonium sulfate solution, and these extracts were combined with the aqueous extract. The growth hormone solution thus obtained was now made up to 3 liters by the addition of 0.1 saturated ammonium sulfate solution and the concentration increased to 0.4 saturation by the addition of 546 gm. of solid ammonium sulfate. After standing overnight (0° to 3°C), the precipitate formed was removed by centrifugation and washed once with 0.4 saturated ammonium sulfate solution, using approximately one liter of the solution. The supernatant solution *I* was saved, to be combined later with other supernatant fluids *K* and *M*.

The 0.4 saturated ammonium sulfate precipitate *H* was next brought into solution by the addition of distilled water and by extraction of the insoluble substance with 0.1 saturated ammonium sulfate, then reprecipitated at 0.4

TABLE 2. GROWTH HORMONE CONTENT OF THE FINAL 0.4 SATURATED AMMONIUM SULFATE PRECIPITATE ('*L*' FRACTION)

Normal Female Rats		Hypophysectomized Rats	
Daily Dose	Gain in 20 Days	Daily Dose	Gain in 20 Days
mg.	gm.	mg.	gm.
83.20	83		
27.70	74		
13.85	72	13.85	69
4.62	59	4.62	65
1.54	42	1.54	61
0.51	25	0.51	51
0.17	10	0.17	46
		0.05	29

saturation in the manner described for the first 0.45 saturated ammonium sulfate precipitate. After this, the process was repeated once more, yielding the final 0.4 saturated ammonium sulfate precipitate *L*. The supernatants *K* and *M* from the precipitates *J* and *L* and their washings were combined with supernatant *I*. These combined supernatants were precipitated by saturation with ammonium sulfate, and the precipitate thus obtained was dialyzed. From the non-dialyzable residue, about 5.0 to 8.0 gm. of solids were obtained; in it were only traces of growth hormone, but large amounts of thyrotropic and gonadotropic hormones and a small amount of lactogenic hormone.

The final 0.4 saturated ammonium sulfate precipitate *L* was washed once with 0.4 saturated ammonium sulfate solution, suspended in water and dialyzed against distilled water in cellophane bags (Visking Corp.) until free from sulfate ion.[4] For assay of growth hormone content the suspension was then dissolved by the addition of dilute sodium hydroxide to pH 9.0 to 9.5. This fraction, referred to, for brevity, as the '*L*' fraction, has proved to be very uniform in its growth-promoting potency. Table 2 gives a growth hormone assay of another typical '*L*' preparation in normal and hypophysectomized rats.

It is interesting to note that the daily administration of as little as 0.05

[4] For dialysis we used a constant flow of cold distilled water in a dializer modified slightly from that described by Kunitz and Simms (11).

mg. of dry organic matter per day of this fraction produced an average growth of more than 1 gm. per day for a period of 20 days in hypophysectomized rats. This preparation is then about as potent as any previously reported.

Table 3 shows the yields of non-dialyzable substance, the relative growth-promoting capacity and the approximate distribution of lactogenic, thyrotropic, gonadotropic and growth hormones in all the fractions obtained during preparation of the particular *'L'* fraction, the growth potency of which is described in table 2. These are figures taken from one preparation, but they are typical. It can be seen that a large amount of material, containing only small amounts of the 4 hormones for which tests were made, is removed at 0.2 saturation

TABLE 3. APPROXIMATE DISTRIBUTION OF PITUITARY HORMONES IN THE PRECIPITATES OBTAINED DURING AMMONIUM SULFATE FRACTIONATION

Fractions	Yield[2] from 2 kg. of Beef Anterior Lobes	Growth Daily Dose of 2 mg. in 20 days	Total Yield of Four Hormones	
			Hormones	Units[3]
0.2 saturated $(NH_4)_2SO_4$ precipitate after washing twice, *C+D*[1]	gm. 83.5	gm. 5	lactogenic thyrotropic gonadotropic growth	700 trace trace 660
Saturated $(NH_4)_2SO_4$ precipitate from supernatant of first 0.45 precipitate, *G*	15.2	3	lactogenic thyrotropic gonadotropic growth	300 1100 >2200 trace
Saturated $(NH_4)_2SO_4$ precipitate of combined supernatants from 2d, 3rd & 4th 0.4 precipitate, *I, K, M*	5.5	10	lactogenic thyrotropic gonadotropic growth	230 11000 5500 640
Final 0.4 saturated $(NH_4)_2SO_4$ precipitate, *L*	36.1	45	lactogenic thyrotropic gonadotropic growth	3000 9000 6000 27769

[1] The letters in italics refer to the fractions outlined on page 486.
[2] Yield, after dialysis, in terms of dry total solids.
[3] Units: *Growth* hormone, growth in normal adult female rats of 40 gm. in 20 days (17 intraperitoneal injections). The units of the other 3 substances are determined in immature pigeons (30-33 days old) given 4 daily intrapectoral doses and autopsied 96 hours after onset of injection. *Lactogenic* hormone, minimum dose which causes appreciable macroscopic growth of crop in 1 of 3 birds. *Thyrotropic hormone*, minimum dose injected which gives histological evidence of thyroid activity in 1 of 3 birds. *Gonadotropic hormone*, minimum dose injected which gives increased testis weight.

with ammonium sulfate. The table shows further that more than 50 per cent of the total amount of thyrotropic and gonadotropic hormones is found in fractions *G, I, K* and *M*, while the greater part of the total lactogenic and growth hormones is concentrated in the *'L'* fraction.

The increase in potency over that of the starting material and the yield of the *'L'* fraction can be seen by comparing these results with the hormone content of the alkaline extract used for the starting material. When the standard alkaline extract was assayed for growth hormone, using normal plateaued female rats, it was found to contain 40,000 U of growth hormone and to have a potency of 1 U per 7.2 mg. of solids. In contrast to this, the *'L'* fraction obtained from it contained 27,769 U, with a potency of 1 U per 1.3 mg. of solids. Thus a 69 per cent yield was obtained and a 5.5-fold concentration effected.

FURTHER PURIFICATION OF THE FOURTH AMMONIUM SULFATE PRECIPITATE ('*L*' FRACTION)

As seen from table 3, the fraction '*L*' containing the major portion of the growth hormone still contains considerable amounts of contaminating hormones. It has not been found possible to remove completely these biologically active contaminants, but some progress toward this end can be reported.

Removal of gonadotropic hormone. The greater part of this hormone can be removed from the growth fraction by utilization of the fact that the gonadotropic hormone is more soluble in dilute or concentrated sodium chloride solution than the growth hormone. There are 3 ways to apply this principle. The first is to dissolve a growth fraction in 5 or 10 per cent sodium chloride solution at pH 6.5 to 8.0, then add hydrochloric acid to the point of maximum precipitation (approximately pH 4.5) and centrifuge. The precipitate that forms is separated by centrifugation. Most of the gonadotropic hormone will be found in the supernatant. Another method is to dissolve the growth fraction in dilute sodium chloride solution, then precipitate the hormone by bringing to saturation with sodium chloride. Still another method is to wash the isoelectric precipitate of growth hormone with 1.0 to 1.5 per cent sodium chloride solution. If it is found that the repetition of one of these methods does not result in a gonadotropic-free growth fraction, one or both of the other methods are applied to remove the remaining gonadotropic hormone. An example is given below.

45 gm. of wet cake (representing 7.3 gm. of organic matter) obtained by filtering off the second 0.4 saturated ammonium sulfate precipitate *H* was dissolved in 3 liters of 10 per cent NaCl solution and precipitated by saturation with solid sodium chloride (pH 6.5). The precipitate was then separated by filtration, redissolved and precipitated two more times. The third precipitate was separated by filtration and dialyzed. After dialysis, the suspended precipitate was dissolved by addition of dilute NaOH solution, iso-electrically precipitated at pH 5.6 and centrifuged. The precipitate was washed 4 times with about 20 cc. of 1.5 per cent sodium chloride solution. The residue was dialyzed, dissolved by addition of NaOH solution and diluted with water to a volume of 160 cc. This solution contained 1.8 per cent of organic matter (as determined by combustion).

When tested in 3 immature pigeons in a total dose of 40 mg. it caused no development of the testis (an organ extremely sensitive to the presence of pituitary gonadotropic hormone). The thyroid showed a minimal response, so a trace of thyrotropic hormone was present. Injected into normal rats, it caused an average increase in body weight of 62 gm. in 20 days at a 10 mg. daily dose.

Removal of lactogenic hormone. Most of this hormone can be removed from the growth hormone fraction in 2 ways. One method takes advantage of the difference in the solubility of lactogenic and growth hormones in 0.1 saturated ammonium sulfate solution. Lactogenic hormone is more soluble in this concentration of salt than the growth hormone, so that when a preparation such as the second 0.4 saturated ammonium sulfate precipitate *H* is extracted repeatedly with small volumes of 6 per cent ammonium sulfate, the lactogenic hormone is extracted, leaving the growth hormone free of this hormone.

In the other method, bromine water is added to the solution of the *'L'* fraction in 0.1 saturated ammonium sulfate. After removing the precipitate which forms, the growth hormone is recovered from the supernatant by saturation with sodium chloride. An example is given to illustrate this procedure.

2500 cc. of the alkaline extract of the *'L'* fraction containing 30.5 gm. of organic matter was used as the starting material. The preparation contained considerable quantities of thyrotropic, gonadotropic and lactogenic hormone. It was precipitated twice by bringing to saturation with sodium chloride. In this way, most of the gonadotropic hormone was removed. The second saturated sodium chloride precipitate was then dissolved in 2200 cc. distilled water and a small amount of dilute NaOH was added to bring the pH to 8.0. 132 gm. of solid ammonium sulfate was added and, with vigorous stirring, bromine water was added to the point of maximum precipitation (about 75 cc.). The bromine precipitate was removed by centrifuging at once. To the supernatant, sodium chloride was added to full saturation, and the precipitate thus formed was removed by filtration and dialyzed. The suspension in the dialysis tube was dissolved by the addition of dilute NaOH solution to pH 9.0, the volume was then adjusted to 710 cc. with distilled water. The analysis of this solution showed 1.28 per cent organic matter. On injection of 3 immature pigeons in a total dose per bird of 51.2 mg., there was no crop gland response or a barely discernible reaction, so that lactogenic hormone had been largely removed. No appreciable increase in testis weight of these pigeons occurred, but the thyroids still showed a response. When this fraction was tested for its growth-stimulating property in normal rats, it gave an average growth of 67 gm. in 20 days at a 12.5 mg. daily dose.

Removal of thyrotropic hormone. Efforts to remove thyrotropic hormone have not been as successful as in the case of lactogenic and gonadotropic hormones, but a few fractions have been made which were devoid of thyrotropic hormone when tested at high doses, and yet were very potent in growth stimulation. It will be noted in the section devoted to removal of gonadotropic hormone that the preparation was very low in thyrotropic hormone; it appears that the thyrotropic hormone, like the gonadotropic, is more soluble in salt solutions than the growth hormone. The following example further illustrates this point.

A non-dialyzed fourth ammonium sulfate precipitate was dissolved in a large amount of 6 per cent ammonium sulfate solution, and the solution brought to pH 4.5 by the addition of 0.2N sulfuric acid. The precipitate was separated by centrifuging, was dialyzed and dissolved by addition of dilute NaOH. Assay in immature pigeons at a total dose of 41.6 mg. showed no evidence of the presence of thyrotropic hormone. Injected in normal rats it gave an average growth of 71 gm. in 20 days at a daily dose of 10.4 mg.

SUMMARY

Methods of assaying the pituitary growth hormone are discussed, and working units are adopted. A detailed description is given of a method of fractionating the standard alkaline extract of the anterior hypophysis by the use of ammonium sulfate. There is effected thereby a simultaneous concentration of the content of growth hormone with reduction in the content of other

hormones. An approximate 70 per cent yield of growth hormone and five-fold increase in potency is obtained. At the same time there is removal of approximately half of the thyrotropic and gonadotropic hormone.

Much, if not all, of the lactogenic hormone found with this procedure to accompany the growth hormone can be removed from such extracts by methods which are given, and methods are likewise offered for the further removal of the thyrotropic and gonadotropic hormones.

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HYPERGLYCEMIA WITHOUT GLYCOSURIA

C. F. DAVIDSON
From the Polyclinic
SEATTLE, WASHINGTON

Hyperglycemia without glycosuria,[1] that is, blood sugar concentration ranging much higher than that generally accepted as normal, unaccompanied by sugar in the urine, has been a frequent encounter in my experience as a result of glucose tolerance tests performed on selected cases. Heretofore, findings of this type appear to have been limited to observations made on old diabetics of long standing. The dearth of discussion of the subject of hyperglycemia without glycosuria among the literature was made evident by a search through Index Medicus from 1932 to 1937 inclusive, which revealed not a single reference under that title.[2]

The following report records cases of hyperglycemia without glycosuria studied during 1936 and the first 10 months of 1937. The work on which the report is based was executed chiefly in the offices of the Polyclinic, located in the American Bank Building, Seattle, and in the Children's Orthopedic Hospital. Occasional studies were made in the Seattle General Hospital, the Swedish Hospital and the Providence Hospital.

METHODS AND RESULTS

Glucose tolerance testing was performed in the following manner. A specimen of venous blood was first obtained in the morning, after the patient had refrained from taking food or drink for 12 or more hours. After such blood specimen was procured, glucose in lemonade or orange juice was administered orally in amounts of 100 gm. for adults, and proportionately less for children. Following administration of glucose, samples of venous blood were taken after 30 minutes, 1, 2, 3, and in some instances after 4 hours. In a few cases of children, in whom vena puncture proved extremely difficult, the micro method was used, capillary blood being obtained by puncturing either the lobe of the ear or the tip of the finger. Sugar concentrations in the venous blood specimens were estimated by the Meyers methods in the studies made in the Polyclinic and Orthopedic Hospital laboratories, and by the Folin-Wu method in the other hospitals. Determination of the color intensity was accomplished by means of a Newcomber colorimeter. (The concentration of glucose is expressed in milligrams per cent; i.e., mg. of glucose per 100 cc. of blood.)

[1] Blood-sugar concentration above 178 to 180 is generally reported as resulting in the spilling of sugar into the urine. The cases reported in this paper, with 3 exceptions, have been confined to those exhibiting a concentration at least 10 per cent greater; i.e., blood-sugar concentration of 200 mg. per cent or higher, without sugar appearing in the urine.

[2] Attention should be called to an article in Canad. M.A.J. 35:298. by C. B. Ward, "The Value of Blood Sugar Studies in Treatment of Damage to the Birth Canal," an analysis of 257 cases.

TABLE 1

Case	Date	Sex	Age yrs.	Ht. in.	Wt. lb.	Glucose, gm.	Blood Sugar values: Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	Complaint
Polyclinic Patients													
P.G.	8/13/37	F	48	65	138	100	126	181	188	200	125	100	Mental disturbance, menopause.
S.W.	10/ 9/37	F	36	63	121	100	128	175	229	135	122	98	Dizzy spells.
B.C.	9/ 3/37	F	25	63	133	100	133	204	200	149	133	98	Asthenia.
H.O.K.	8/ 2/37	F	62	64	140	100	222	212	185	156	144	64	Spells of amnesia, hypothyroidism.
G.W.	8/ 4/37	F	70	67½	175	100	169	200	188	217	125	108	Arthritis.
C.W.C.	7/ 2/37	F	69	63	142½	100	114	116	227	161	117	92	Meniere's disease.
J.D.D.	4/14/37	F	51	67	220	100	144	185	224	131	89	76	Obesity, painful joints.
L.H.	3/17/37	F	50	66	231	100	188	238	229	140	181	163	Obesity, rheumatism.
G.M.C.	9/ 4/36	M	67	66	154	100	119	196	208	135	82	75	Tremor suggestive or Parkinson's disease.
E.C.K.	7/31/36	F	56	63	139	100	139	208	181	117	62	59	Stiffness, numbness in hands, face; spells of aphonia.
L.D.L.	7/28/37	F	22	64	214	100	104	181	200	166	104	82	Obesity.
G.G.	3/18/36	M	8				151	208	144	117	128	111	Polysinusitis.
G.B.	3/25/36	F	13	63	134	100	153	238	238	176	142	138	Obesity, fainting spells, petit mal with menses.
E.F.	11/18/36	F	7	50½	75½	100	147	198	128	100			Obesity.
A.C.B.	4/ 1/36	F	45	64	174	100	142	200	161	117	109	119	Mental instability, menopause.
C.E.	11/ 9/36	M	15	68	131	100	126	222	153	93	106	73	Fainting spells, epileptiform.
P.P.	7/15/36	F	26	66	110	100	140	232	153	104	98	100	Lack of endurance
S.S.	9/ 9/36	F	28	62½	112	100	149	175	200	128	125	133	Defective peridental tissue.
R.E.	11/10/36	F	24	64½	120	100	200	185	131	156	104	97	Amnesia.
D.J.	11/14/36	F	10	59	104	100	128	200	172	172	87	76	Obesity.
R.P.	5/16/36	F	36	65	140	100	129	181	192	142	116	100	Double antrum infection.
E.J.S.	12/ 7/36	F	58	66	148	100	125	120	200	142	160	90	Undue fatigue.
L.W.	12/ 5/36	F	26	65½	115	100	126	200	142	98	72	84	Undue fatigue.
B.S.	12/15/36	F	8	51½	81		129		250	181	88.5		Obesity suggestive of basophil adenoma.
C.N.	9/25/36	F	29	64	155	128	164	234	197	208			Increasing weight, pain in back.
Orthopedic Out-Patients													
K.H.	6/ 4/37	F	11	56	130	100	174	214	187	166	150		Obesity suggestive of basophilism.
J.M.	2/12/37	F	5	58	77	61.6	192	194	168	140	138		Bilateral congenital cataracts.
E.S.	10/29/37	F	12	55½	92½	73	133	257	223	135	156		Overweight following mastoidectomy.
F.C.	8/25/37	F	13	58	89	71	144	187	262	230	176		Mental retardation.
B.S.	8/26/37	F	13	49	56	42	144	272	400	187	187		Stunted development, dwarfism.
W.P.	9/27/37	M	12	60	131	100	182	166	208	174	170		Recurring asthma.
O.D.	4/30/37	M	12	60½	147	100	138	200	182	176	166		Fröhlich's syndrome.
M.J.B.	10/ 1/37	F	13	59	89½	71	187	250	300	185	246		Mental dullness, poor posture.
P.M.	9/ 2/36	F	7	46	52	42	166	230	158	150			Obese and unsocial.
B.W.	7/ 6/36	F	12	62	197	156	180	276	182	157			Obesity and overgrowth.
E.J.	7/30/37	M	10	55	74	59	157	214	174	157	200		Prone to exhibit colds and disability.
J.D.	7/19/37	F	8	53	97	78	168	214	250	151	148		Overweight.
B.H.	4/16/37	M	12	58	131	100	145	272	180	144	141		Obesity, typical Fröhlich's.
B.P.	6/ 6/36	F	12	65	142	112	161	217	178	156			Persistent pain at site of fractured ankle of 5 years' standing.
	10/30/36					100	176	272	166	187	157		
	1/15/37					100	145	217	148	153	168		
T.S.	11/ 6/36	M	12	68½	120	100	151	238	187	174	161		Overgrowth, easy faitgue.
L.S.	7/23/36	M	9	61½	96	80	150	223	150	174			Mental retardation.
M.S.	8/ 4/36	F	13	62	121	96	138	250	148	147	145		Obesity.
E.S.	2/24/37	M	6½	50½	86	68	141	272	250	187			Obesity of pituitary distribution.
S.A.H.	8/ 6/37	F	6	40	30	24	107	150	214	172			Stunted growth, development.
P.O.K.	8/12/37	F	8	51	81	72	187	172	169	163	153		Obesity and hiccough.
J.R.	10/ 1/37	M	3	38	34	26	150		214	250			Defective teeth, limited speech and muscular instability.
D.S.	9/17/37	F	10	59	125	100	131	168	164	192	200		Obesity, painful feet.
Orthopedic In-Patients													
J.F.	10/11/37	F	18	20	22	21	166	275	275	250			Muscular atony or dystrophy.
E.D.	2/ 5/37	M	13	62	117	94	166	250	172	180	164		Old Frölich's, epileptiform convulsions 10/26/33.
	10/26/33					68	83	148	81	75			
J.A.	9/17/37	F	13	60	89	72	147	220	144	131	133		Admitted for study on account of menstrual pain.
R.S.	2/27/36	M	3½	39	53	42	150	176	272	166			Mental deficiency.
J.F.	11/ 6/36	M	10	55	71	57	153	250	238	196	150		Instability and asthenia.
L.D.	9/27/37	M	12	55½	73	62	151	242	157	115	114		Epileptiform seizures, retarded mental development.

Case	Date	Sex	Age yrs.	Ht. in.	Wt. lb.	Glucose, gm.	Blood Sugar Values					Complaint
							Fasting	½ hr.	1 hr.	2 hr.	3 hr.	4 hr.	
D.H.	10/16/36	F	12	62	152	100	147	182	147	242			Pain in abdomen, body discomfort sufficient to keep her out of school.
	11/14/36					100	176	300	375	425	200		
	11/18/36					100	107	220	208	211	189		
	11/23/36					100	166	250	425	400	333		
	11/30/36					100	205	250	300	300	300		
J.M.	7/26/37	M	13	42½	40	34	107	220	214	153	127		Moderately obese, mentally retarded.
V.B.	9/ 9/36	F	13	63	139	100	182	200	192	166			Epiphysitis, lumbar spine, pain in legs.
	10/30/36					100	182	192	148	180	138		
	2/27/37					100	153	200	153	151	142		
E.B.	12/16/36	F	7			32	136	166	250	230	187		Ossium fragilitas.
Z.C.	12/11/36	F	6	41	38	30	200	300	214	185	151		Growth impeded after stopping thyroid 10/18/34.
G.F.	6/26/37	M	13	59½	75	60	163	217	223	211	120		Overgrowth, precocious sex development, malnutrition.
L.F.	8/ 6/37	M	13	63	163	100	166	230	214	166	151		Overgrowth, slow in school, stomatitis for 2 years.
E.G.	2/ 5/37	F	10			67	133	197	300	272	257		Jaundice, post mortem diagnosis: cirrhosis of the liver.
M.E.G.	2/ 4/37	F	8	49	61	49	197	246	272	189	172		Numerous areas of osteomyelitis of the skull.
P.K.	3/ 1/37	M	9	50½	97	77	192	272	238	174	164		Osteochondritis of both hip joints.
N.S.	4/ 5/37	F	6	48	55	50	170	238	205	182	119		Perthe's disease?
G.S.	8/ 6/35	F	10	57	105		90.9	217	338	147			Mentally slow, obese.
	1/25/36					80	159	189	214	150			
	8/18/36					85	200	250	272	300			
	9/ 8/36						180	250	242	217			
P.A.M.	2/17/36	F	3			20	120	176	333	237			Died of bronchopneumonia, no autopsy. Tonsillectomy 2 months previously.
S.H.	8/16/36	F	13				150	230	214	176			Severe headache, malaise.
L.W.	7/ 9/36	F	6	41	35		132	238	214	151			Slow mentally, poor coördination. Small for age. Gait of 2 year-old child.
	11/18/36					30	120	157	200	123	127		
	11/23/36					30	148	187	220	176	157		
	12/ 2/36					30	157	214	365	300	136		
	12/ 7/36					41	159	235	255	250	161		
	12/14/36						129	190	131	140	140		
J.B.	8/18/36	F	9	54	113	90	187	223	220				Obesity.
Swedish Hospital Patient													
E.C.	11/ 7/36	F	29			100	85	134	181	212	151		Delayed healing of operative wounds (hallux valgus, bilateral).
Providence Hospital Patients													
D.C.	10/31/36	M	14	62	166	100	141	300	250	214	80		Fröhlich's syndrome. Slipped epiphysis, rt. hip.
	11/23/36					100	100	142	180	136	116		
	11/30/36					100	104	217	206	130	108		
	12/ 9/36					100	106	106	96.6	86	84		
	12/21/36					100	108	130	112	105	100		
J.Mc.		F	65			100	118	206	260.8	276	276		Paralysis, cerebral hemorrhage.

Repeated examinations, in the few cases where they have been performed, would indicate that the condition tends to be a persistent one. Repeated examinations in all cases would have been advantageous to their clinical care, and might have offered added conviction to this presentation. However, various factors combined to preclude consistent application of this procedure, the expense involved, the press of new work, i.e., more work than could be accomplished by the limited personnel in the laboratories, and the more or less insistent demand that therapeutic measures be initiated.

The data in table 1 give the date of study, sex, age, height and weight in the majority of cases, the amount of glucose fed, blood-sugar values, and a brief statement of the complaint at the time of study. It should be emphasized that none of these patients showed sugar in the urine.

The work in each of the laboratories was executed by trained laboratory assistants, supervised by trained medical men. The technic and reagents in the Polyclinic laboratory and Orthopedic Hospital laboratory, where most

of the studies were done, have been checked and double-checked. The competence and dependability of the work in the various laboratories is further substantiated by the fact that, during 1936 to 1937, the Orthopedic Hospital laboratory found fasting blood-sugar levels in 11 tests on known diabetics to range between 150 to 600 mg. per cent. Two patients manifesting hyperglycemia on glucose tolerance testing exhibited sugar in the urine, and 6 patients tested yielded concentrations within normal limits. The Polyclinic laboratory, during the same period, made 204 glucose tolerance tests, which revealed hyperglycemia without glycosuria in 30 cases, hyperglycemia with glycosuria in 22, and glycosuria without hyperglycemia in 5 cases. The study on one patient in the Swedish Hospital yielded findings interpreted as indicating Islands of Langerhans adenoma, the presence of which was subsequently confirmed by operation. This case is cited for the outstanding evidence it bears of the dependability of the laboratory findings.

Interest in the prosecution of the study of hyperglycemia without glycosuria has been provoked by contact with *a*), cases in which ordinary examinations failed to reveal causes to which the clinical manifestations might be ascribed; *b*), cases in which therapeutic measures directed at ascribed causes had failed to give clinical improvement. This interest has been maintained by experience with cases in which, after hyperglycemia without glycosuria had been demonstrated, clinical improvement followed therapeutic measures directed at this deviation in metabolism. Experience through 10 years of study and treatment along such lines has led me to believe that the patient exhibiting hyperglycemia without glycosuria is subject to the same vicissitudes as the patient exhibiting hyperglycemia with glycosuria.

SUMMARY

Hyperglycemia without glycosuria has been revealed by means of glucose tolerance testing in an amazingly high percentage of cases, in which the author's judgment has deemed a study of endocrine balance to be indicated.

CLINICAL AND LABORATORY NOTES

THE TECHNIC OF ADRENALECTOMY IN DOGS

J. FREUD, INA E. UYLDERT AND M. L. WATERMAN*

Numerous writers have described technics for adrenalectomy, but little stress has been laid upon preserving the integrity of the splanchnic nerves. Having had occasion to use many adrenalectomized dogs in the assay of various adrenal preparations, we became dissatisfied with the operative technic previously employed, and were led to evolve a more satisfactory method, which is described herewith. It is now routine in our laboratory.

Sequelæ of adrenalectomy. Between 1932 and 1935, 56 dogs of mongrel varieties were adrenalectomized in this laboratory, of which only 24 survived, 32 dying within the 48 hours following one-stage bilateral adrenalectomy. The two-stage operation was totally unsuccessful. Death, which was preceded by drowsiness, retching and vomiting, muscular weakness, slow respiration and pulse rate, and rapid fall of temperature, could be postponed 2 to 4 hours at most by the intravenous injection of NaCl, $NaHCO_3$ and glucose, or by Ringer's solution. In one dog in adrenal insufficiency, 100 mg. cystein and 120 mg. glycocoll, both 20 mg. at a time, were injected over a period of 9 hours. Every injection improved the general condition of the dog, but 2 hours after the last injection the dog died. In another case this therapy had no effect. After the saline injection, the animals always revived for a short time. Injections of cortin (up to 80 cc. subcutaneously or intravenously) were without effect. As soon as 150 cc. of saline has been injected into a dog of 8 to 10 kg. body weight, the animal will lift his head, and later on it is able to stand, and then walk again, but some hours later it will die. Nevertheless those dogs that survived adrenalectomy and were treated with NaCl and cortin for a few weeks developed symptoms of true insufficiency when treatment was suspended.

True adrenal insufficiency. The symptoms occurring in true adrenal insufficiency are similar to the symptoms described above, except that they appear much more slowly, i.e., within 4 days instead of 4 hours. On the first day, food is taken and the temperature is normal (about 38.5° C). On the second day there is partial refusal of food, vomiting, and a fall in temperature (to 37.8° C). On the third day there is a complete refusal of food, vomiting, and a fall in temperature to 37° C, and on the fourth day the rejection of food and the vomiting is followed by diarrhoea, and a fall of temperature to 36.6° C. Death may then occur, and a fairly reliable symptom of impending death is a cold gingiva. If the gingiva is still warm, a further fall of temperature to 34° C is possible, and the dog may die the next day. Adynamia, as seen from the abnormal gait, may begin on the first day, at any time, or not at all. Diarrhoea frequently occurs the day before death, and blood may appear in the feces. At autopsy, erosions or ulcers are occasionally found in the gastric mucosa. The intestinal mucosa is sometimes very congested and hemorrhagic.

Saline infusions given on the fourth day prevent cortical insufficiency for a while

* From the Pharmaco-Therapeutic Laboratory, University of Amsterdam, Amsterdam, Holland.

and subsequent cortin therapy will remove the other signs of cortical insufficiency. No dog can definitely be saved from insufficiency without cortin. Since the dogs take potassium salts with their food, we were not able to keep alive adrenalectomized dogs merely by administering NaCl and sodium citrate. When a therapy with cortin and NaCl is begun early, i.e., on the second or third day, the symptoms disappear still more rapidly, and the dog becomes healthy again. The symptoms are only incurable when cortin is given too late, for example, 15 min. before impending death.

The splanchnic plexus. The symptoms which follow adrenalectomy so resemble those of splanchnic lesions, described in the literature, as to suggest that an inadvertent splanchnic lesion might account for the poor results obtained. After removal of the coeliac plexus in the dog, Popielski[1] observed hemorrhagic diarrhoea accompanied by gastro-intestinal congestion and gastric and duodenal ulcer at autopsy, results which were not obtained by mere severance of the splanchnic nerves.

According to Stiemens,[2] the adrenals of the dog are innervated by branches of the ganglion solare and intermedium; the coeliac plexus is situated close to the adrenal, and may even be adherent to its connective tissue. On the left side, the renal ganglia 1 and 2 communicate directly with the superior mesenteric ganglion, the coeliac plexus and the ganglion intermedium, and are so situated on the dorsal surface of the adrenal gland that one might say the latter is resting on the ganglia, to each of which it is connected by numerous nerve fibres. On the right side, the ganglia are not so close to the gland. By means of an elaborate anatomical dissection, one of us (M. L. W.) confirmed Stiemens findings, wherefrom we concluded that our original operative technic had necessarily entailed severe damage to the ganglionic plexus of the suprarenal region and more particularly to the coeliac, superior mesenteric and renal ganglia.

A modified operative technic which should avoid ganglionic trauma was based on these findings.

Improved technic. The procedure of the modified two-stage operation follows. The skin having been prepared by cutting the hair and sterilizing with picric acid, the dog is anesthetised with morphine (5 mg. per kg. body weight) and warm ether insufflation (5 to 10 cc. per kg.), and a 3-inch incision is made from the costovertebral angle parallel to the last rib. A one-inch incision is then made into the tight fascia of the external oblique muscle, long enough to admit one finger of each hand, and enlarged by traction until a soft bulge of peritoneum is felt across the bottom of the wound. The kidney and adrenal are exposed by opening the peritoneum. The large lumbar or suprarenal vein, which usually crosses over the ventral surface of the adrenal and leads to the vena cava over the adrenal, is dissected free and doubly ligatured. The gland is cautiously shelled out of its capsule by the aid of sticks wrapped at each end with cotton wool, thus avoiding damage to the capsule and ganglia. The peritoneal and muscular layers are closed with catgut, and the skin incision with silk. Finally the skin is painted with iodine and left without a dressing. Full aseptic precautions are observed throughout.

Results of the new technic. Of 30 dogs adrenalectomized by 3 different operators, 20 survived longer than 48 hours, and most of these were used for cortin assay for several months or years. Of the 10 which died within 48 hours, 4 showed hemorrhage general or limited to the adrenal region; 2 pneumonia; 2 unintentional damage to the regional ganglia; and the remaining 2 failed to suggest any error of technic. Where splanchnic trauma is avoided, the mortality from two-stage adrenalec-

[1] Popielski, I.: Arch. f. Anat. u. Physiol. p. 338. 1903.
[2] Stiemens, M. J., Verhand, Kon. Akad. Wetensch. Amsterd. 2:33. 1934.

tomy is evidently much reduced. In our earlier series, death nearly always followed the removal of the second adrenal, although the first stage had not been performed with any particular attention to the splanchnic plexus. Therefore, death clearly depended on the adrenal and the splanchnic trauma together, not on any non-specific operative trauma.

The improved technic did not enable us to perform one-stage bilateral adrenalectomy with success; in such an attempt 3 out of 3 dogs were lost within 24 hours of the operation.

The average time required between the first incision and the last suture has been increased from 20 or 30 minutes to 30 or 90 minutes; better results from the long operation show that the actual time spent is unimportant. Rogoff and Stewart[3] emphasize the necessity for speed and skill if shock is to be avoided, but our results show that the only special skill required is in the avoidance of the splanchnic ganglia. Two of the 20 surviving dogs were without either cortin or NaCl for the first 2 days, their condition remaining excellent until the third day, when one of them showed mild symptoms of insufficiency.

Bilateral splanchnicotomy was performed on 3 dogs, the ganglia being removed and the adrenals left intact. The animals tolerated the actual operation well, but were in bad condition 6 hours later. The first, killed by morphine and chloroform on the ninth day, showed neither congestion of the intestinal mucosa nor ulceration of the stomach; the second, killed after only 2 days by 20 per cent $MgSO_4$ intravenously showed multiple erosions of the gastric mucosa without intestinal congestion; the third, killed after 24 hours by morphine and $MgSO_4$, showed changes similar to the second. If the first also had had gastric ulcers, these must have healed in the course of 9 days. Freud, Luwisch and Oestreicher[4] described the occurrence of ulcers in dogs adrenalectomized by the old method, which ignored the splanchnic nerves.

Where there is true adrenal insufficiency and intact ganglia, such ulcers may (as in the case of 3 dogs) or may not (as in the case of 2 dogs) be present at autopsy, and of 7 adrenalectomized dogs that died from causes other than adrenal insufficiency 1 had gastric ulcers. Apparently these ulcers may be a sequela of either splanchnic, cortical or combined lesion, which may provide the theoretical basis for an experimental cortin therapy of (acute) gastric ulcer and perhaps also of ulcerative colitis.

Summary. Injury to splanchnic ganglia, in the course of a total ablation of adrenal tissues, is lethal to the majority of dogs. Injury to the splanchnics in the course of a unilateral adrenalectomy is not lethal so long as the second adrenal is left intact. Adrenalectomy performed with conscious avoidance of trauma to the splanchnic nerves was practically always successful. We were unable to perform bilateral adrenalectomy in one stage without fatal sequelae. Adrenalectomy, performed without conscious avoidance of trauma to the splanchnic plexus, had fatal sequelae in a majority of instances, whereas the symptoms leading to death within 48 hours are similar to those which develop in successfully adrenalectomized dogs deprived of cortin although alleviated for a few hours by intravenous injections of NaCl and $NaHCO_3$; ultimately they are lethal in all cases. This holds true for dogs maintained in good condition with cortin and diet, which are deprived of treatment until they have passed into an irreversible state, i.e., shortly before death. Gastrointestinal erosions and ulcers bear some relation to the pathology of the adrenals and splanchnic nerves.

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[3] Rogoff, J. M., and G. N. Stewart: Am. J. Physiol. **78**:683. 1926.

[4] Freud, J., D. Luwisch and F. Oestreicher: Brit. M. J. **1**:1216. 1935.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Arthur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

A six-hour assay for the quantitative determination of estrogen. Astwood, E. B. To appear in this Journal.

Certain early effects of estrogen on the uterus of the immature rate are described. Following a single injection of 0.1γ estradiol there is a prompt increase in uterine weight which reaches a maximum of 60% in 6 hours, followed by a fall and a rise to a second maximum at 30 hours. The 6-hour weight increase is accompanied by, and almost entirely due to, an increase in the water content of the uterus. This water is accumulated chiefly in the stroma of the mucosa, and to a lesser extent in the subserosa and between the muscle layers. Using this 6-hour maximum as a time interval, a standard curve has been constructed relating uterine weight response to estradiol dosage. From 0.006γ to 0.1γ estradiol a direct logarithmic relationship has been established between dosage and uterine response. Based upon this, an assay method has been evolved permitting the accurate assay of estrogenic substances in 6 hours. Tests are performed by injecting dilutions of the unknown solution into groups of 21 to 23-day old rats, and determining the average uterine weights 6 hours later. From the weight increase, the estrogenic potency of the solution is read directly from a standard curve. The test has been standardized with estradiol and estrone so that assay results can be expressed directly in I.U.

The occurrence of coitus-induced ovulation in the adrenalectomized rabbit. Brooks, C. McC., and S. W. Page. To appear in this Journal.

Removal of both adrenal glands within 15 to 45 minutes after mating did not prevent ovulation in 5 female rabbits. All of these animals died within 2 to 6 days following the operation, presumably from cortical deficiency. Five completely adrenalectomized rabbits mated on the second or third day after adrenalectomy and ovulated normally. These animals were injected with Progynon-B and fed large amounts of sodium chloride in an effort to delay the development of cortico-adrenal deficiency symptoms. Progynon-B and the high salt diet did not produce ovulation in unmated rabbits. It was concluded that the adrenal glands are not an essential link in the chain of events whereby coitus stimulates the ovulation-producing activity of the anterior lobe of the rabbit's pituitary.

Fate of ova accelerated in their rate of passage through the fallopian tubes of mice by massive injections of an estrogen. Burdick, H. O., and R. Whitney. To appear in this Journal.

Acceleration of the rate of passage of ova through the fallopian tubes of mice by massive injections (100 to 500 R.U.) of the estrogen progynon-B results in degeneration of the fertilized ova. This deleterious effect upon the ova appears to be due to a lack of a proper sustaining fluid in the uterus, and not to the direct action of progynon-B. Acceleration usually occurs within 12 to 30 hours after injections. Ova in early cleavage stages show definite pathological changes within 24 hours after entrance into the uterus. These massive injections of progynon-B will prevent pregnancy whether or not acceleration of the rate of passage of fertilized ova has been accomplished.

Pituitary extracts in Simmonds' Disease. Dick, G. F., and W. C. Dine. To appear in this Journal.

Two cases of hypo-pituitary syndrome are reported. Both cases received various pituitary extract at intervals over long periods of time. In one case the basal metabolic rate was raised by administration of growth and gonadotropic factors, and a fall in the rate was produced by administration of Lugol's solution. Both cases seemed to do better clinically with growth and gonadotropic factor, but there was little laboratory evidence to support this.

The purification of the anterior pituitary growth hormone by fractionation with ammonium sulfate. Evans, H. M., N. Uyei, Q. R. Bartz and Miriam E. Simpson. To appear in this Journal.

Methods of assaying the pituitary growth hormone are discussed, and working units are adopted. A detailed description is given of a method of fractionating the standard alkaline extract of the anterior hypophysis by the use of ammonium sulfate. There is effected thereby a simultaneous concentration of the content of growth hormone with reduction in the content of other hormones. An approximate 70% yield of growth hormone and 5-fold increase in potency is obtained. At the same time there is removal of approximately half of the thyrotropic and gonadotropic hormone. Much, if not all, of the lactogenic hormone found with this procedure to accompany the growth hormone can be removed from such extracts by methods which are given, and methods are likewise offered for the further removal of the thyrotropic and gonadotropic hormones.

Cytologic evidence of the gonadotropic activity of the rabbit's anterior hypophysis. Friedgood, H. B., and A. B. Dawson. To appear in this Journal.

The pituitary glands from 114 female rabbits were sectioned serially after formalin-mercury bichloride fixation. The control group consisted of 61 rabbits in 7 different physiological states. The experimental series included 53 does, 50 of which were killed at specified intervals after mating. Three were autopsied late in pseudopregnancy. Isolated, small carmine-staining cells can be found in the pituitaries of castrate and immature rabbits. They contribute insignificantly to the cytological make-up of the anterior pituitary gland in such animals. Their number appears to be increased in animals approaching sexual maturity, and may occasionally be fairly prominent in the pituitary of the estrous rabbit. These cells really dominate the cytological appearance of the gland only after mating. They become markedly enlarged and increased in number. The size, density and coarseness of granules are markedly increased; and the cells take a deeper, more striking carmine stain. Usually they are not uniformly distributed throughout the pituitary, but tend to become aggregated into circumscribed areas which are sharply localized. Rapid functional changes take place in the anterior pituitary within the first few hours after mating. It is known that within 1 to 2 hours postcoitum the blood stream receives enough gonadotropic hormone from the anterior pituitary to produce ovulation. The carmine cell is a prominent feature of the anterior pituitary during this phase of the gland's gonadotropic activity. Its precise relation to the pituitary hormones which are known to be secreted at this time is as yet not clear.

Ovaries secrete male hormone. IV. Effect of ovarian androgens on accessory size in the mouse. Hill, R. T., and M. T. Strong. To appear in this Journal.

Evidence is presented which shows that androgens, coming from ovaries grafted in the ears of male mice, are independent of testis function with respect to their action on sex accessories. It is felt that this evidence is suggestive of a chemical difference between these two sources of accessory stimulating substances. Irrespective of this difference, a similarity or identity of physiology exists in the two substances.

Pituitary Emaciation. Kunstadter, R. H. To appear in this Journal.

There are certain cases of pituitary emaciation that may be distinguished from true Simmonds' disease. The disease is characterized by its predilection for adolescent girls, and is probably due to a hormonal suppression of the intact anterior lobe of the hypophysis. Characteristically there is anorexia, marked loss of weight occurring suddenly or gradually in a girl who has apparently been in good health. The failure of the menstrual period to appear, or the development of amenorrhea and oligomenorrhea, are characteristic. The secondary characteristics are delayed, or in the already sexually mature, the breasts and genitalia atrophy.

The severe symptoms and signs of Simmonds' disease are not present. The course is favorably influenced by endocrine therapy. Two cases have been reported.

Pituitary dwarfism: case report illustrating response to treatment. Lawrence, C. H. To appear in this Journal.

A case is reported illustrating the effect of treatment in a boy 17 years and 4 months old, exhibiting sexual infantilism and dwarfism of the eunuchoid type. An increase of 6½ inches in height, and normal sexual development occurred during the period of treatment. The reasons for ascribing these results to treatment rather than to spontaneous growth and development are briefly stated. Photographs, a chart and a table of measurements are appended.

Effect of Coitus on Gonadotropic Content of Pituitary Glands of Pseudopregnant Rabbits. Makepeace, A. W., G. L. Weinstein and M. H. Friedman.

Normally following coitus, an estrous rabbit ovulates. A pregnant or pseudopregnant rabbit will frequently accept coitus but does not ovulate. Coitus and ovulation in the estrous rabbit is followed by a rapid depletion of the gonadotropic material in the pituitary gland. Coitus in a 9-day pseudopregnant rabbit is not followed by such pituitary depletion. Therefore, failure of ovulation in a rabbit during pseudopregnancy is caused by some interference with pituitary discharge.

Nutritional requirements of pregnant and lactating rats studied by the self-selection method. Richter, C. P., and B. Barelare, Jr. To appear in this Journal.

Ten female rats were offered a choice of the following substances in separate containers: casein, sucrose, olive oil, sodium chloride, dibasic sodium phosphate, calcium lactate, potassium chloride, dried Baker's yeast, cod liver oil, wheat germ oil, and water. These animals showed normal growth and regular 4 to 5-day estrous cycles. After mating they gave birth to normal litters and nursed them until weaning time. It was noted that the animals ingested large amounts of fat and much smaller amounts of carbohydrate and protein. During pregnancy and lactation, the fat consumption increased, as did the protein in less degree, whereas the carbohydrate intake remained on a fairly constant level. Striking increases were noted in the intake of sodium chloride, calcium lactate, and dibasic sodium phosphate. The potassium chloride appetite showed no great change. Wheat germ oil and cod liver oil intake also remained essentially the same. The daily caloric intake increased during pregnancy and lactation, reaching a level almost 3 times that of the pregestational period. Fewer calories per day were ingested than on the McCollum diet. The solid food intake in grams (including the oils) was much lower than that noted on the McCollum diet. The fact that the animals grew and reproduced with as great success as on the McCollum diet, in spite of the lower solid food and caloric intake, is evidence of the efficiency of the self-selection method in the rat.

Clinical indications for A.P.L. hormone. Schaefer, R. L., E. A. Sharp and James V. Lammy. To appear in this Journal.

It is the author's premise that the anterior-pituitary-like sex hormone is a maturing factor of the gonads, and that any clinical condition representing an immature state of the gonads is a therapeutical indication for its administration. Sixteen cases of genital hypoplasia and cryptorchidism, 15 cases of amenorrhea and 13 cases of menometrorrhagia were treated. Results were uniformly good where treatment was indicated, with the exceptions cited in the text. Competent diagnostic procedures should be instituted to rule out organic disease and also to determine those findings which indicate gonadal immaturity. This will serve the purpose of early recognition, which is of utmost importance in correcting preadolescent endocrine imbalances. Pluriglandular treatment is frequently indicated. Secondary thyroid deficiency in this locality can rarely be disregarded. Definite dosage cannot be defined but must be individualized, and thereby adequate to bring about the expected biologic result. Treatment should be prolonged and continuous before concluding that the existing imbalance cannot be corrected.

Naso-genital relationship: Induction of pseudopregnancy in rat by nasal treatment. Shelesnyak, M. C., and S. Rosen. To appear in this Journal.

The periodicity of estrus cycles of 209 young adult female rats was observed. Of this group, 185 were employed to examine the nature of estrus cycle after application of various agents to the nasal mucosa. Anesthetization of nasal mucosa with nupercaine produced pseudopregnancy in 58 of 115 animals. Controls which received buccal applications, or nasal manipulations without the agents, were negative.

Studies on the pituitary weight of rats inoculated with a transmissible tumor. Twort, J. M., and M. Lasnitzki. To appear in this Journal.

A number of rats previously inoculated with Jensen rat sarcoma were divided into two groups according to receptivity. Each group was further divided into sub-groups according to brain weight, the sub-groups being in turn separated into males and females. It was found that, on an average, the ratio of pituitary dry weight to brain weight was usually smaller in the groups of animals bearing tumors than in those without. When all the animals of each sex were massed, the average decrease was about 23% among the males, and about 17% among the females. It is suggested that a connection exists between the diminution of the pituitary gland and the increase in the size of the spleen observed in tumor-bearing animals. In every group, except one, the relative weight of the female pituitary was found to be greater than that of the male organ. On an average, the increase was about 45% among the tumor-bearing animals, and about 34% among the tumorless animals.

Acceleration of the rate of passage of fertilized ova through the fallopian tubes of rabbits by massive injections of progynon-B. Whitney, R., and H. O. Burdick. To appear in this Journal.

Single injections of 5000 R.U. of the estrogen, progynon-B, given after ovulation results in the acceleration of the rate of tubal passage of the fertilized ova of rabbits. These accelerated ova in early cleavage stages disintegrate within a few hours after exposure to the uterine fluids and pregnancy is therefore prevented. These results coincide with those of similar experiments with mice.

ADRENALS

The blood volume regulatory mechanism in adrenalectomized animals (Sur le mécanisme régulateur du volume sanguin chez les animaux surrénalectomisés). Gradinesco, A., and N. Santa, Compt. rend. Soc. de biol. **125:**195. 1937.

A note in which it was stated that escape of blood plasma into the tissues (vasodiaplasmie) was originally observed in adrenalectomized animals by Gradinesco in 1913 when it was associated with adrenalin insufficiency. Recent studies, however, have indicated that this condition is due to adrenal cortical insufficiency but that it can be prevented apparently by a substance different from the life-sustaining adrenal cortex principle.—E. Cutuly.

Physiological properties of adrenal cortical extracts (Sur les propriétés physiologiques des extraits corticosurrénaux). Gradinesco, A. and N. Santa, Compt. rend. Soc. de biol. **125:**197. 1937.

Adrenal cortex extracts prepared by the method of Swingle and Pfiffner were said to contain a second factor in addition to the life-sustaining principle. In dogs this second factor prevented the escape of blood plasma into tissues. In adrenalectomized dogs and cats, adrenal cortical extract in doses sufficient to maintain life had no action on blood pressure; large doses caused slight vasoconstriction. The cortical extract also had a marked mydriatic action on enucleated eyes of tadpoles.—E. Cutuly.

The distribution of body water and electrolytes in adrenal insufficiency. Harrison, H. E., and D. C. Darrow, J. Clin. Investigation **17:** 77. 1938.

Following adrenalectomy, rats show diminished serum sodium and chloride, with increased potassium and N.P.N. The sodium and chloride changes are less marked than in dogs, cats, or men. High intake of potassium accelerates the onset of insufficiency symptoms in adrenalectomized rats. Insufficient rats have elevated muscle cell potassium, but normal liver cell potassium; treatment with cortex extract or with saline returns the values to those of controls. In all classes of animals studied, the water in the cells varied inversely with extracellular sodium. Cortex appears to influence water movement only as it influences the extracellular sodium concentration. Adrenal insufficiency symptoms in rats are not related to shifts in water distribution.—E. L. S.

Potential changes in the frog's adrenal induced by illumination of the body. (Ueber Potentialänderungen an der Nebenniere des Frosches bei Belichtung des Körpers). Hasama, B., Arch. f. d. ges. Physiol. **238:**758. 1937.

The potential changes occurring in the frog's adrenal were recorded with a unipolar electrode. Illumination of the body or the injection of nicotine resulted in an increase in the

negative potential of the gland. This electric change is attributed to the liberation of epinephrine, which is also held responsible for the observed contraction of the melanophores.—A.G.

Use of dogs with sectioned spinal cords to detect sympathin in the blood (De l'utilization du chien 'sans moelle' pour la mise en evidence de la sympathine dans le sang). Hermann, H., G. Morin, F. Jourdan, and J. Vial, Compt. rend. Soc. de biol. **125**:287. 1937.

In dogs following destruction of the spinal cord, up to the first thoracic nerve and bilateral adrenalectomy electrical stimulation of the splanchnic nerve trunk causes markedly increased arterial pressure; the tonus of the blood vessels of the denervated kidney, however, is but slightly affected. If cocain is administered to such faradically stimulated denervated dogs, the vessels of the kidney undergo definite vasoconstriction. The explanation offered is that cocain not only sensitizes the animal to sympathin, but also causes peripheral vasoconstriction, thus permitting sympathin demonstrably to manifest its constrictor action on the larger vessels.—E. Cutuly.

Regeneration of nerves to the adrenal gland. Hollingshead, W. H., and H. Finkelstein, J. Comp. Neurol. **67**:215. 1937.

Denervation of the adrenal gland has been obtained by removal of the lower thoracic and upper lumbar sympathetic chain. Histological study of the adrenals of animals sacrificed 1, 2, 3 and 4 months after operation has indicated a gradual return of the innervation to the gland. The medullary plexus was found to be re-formed in an apparently normal manner as early as the fourth month after operation. The regenerating fibers arose from lower thoracic and upper lumbar spinal nerves.—Authors' Abts., Wistar Bib. Service.

The action of estrin on the adrenal. The phagocytic symplasm in the mouse (A propos de l'action de l'oestrine sur la surrenale: les symplasmes phagocytaires chez la souris). Lacassagne, A., and A. Raynaud, Compt. rend. Soc. de biol. **124**:1183. 1937.

In mice adrenals there develops normally a non-cellular phagocytic zone just outside the medulla. This zone develops more rapidly and extensively in animals receiving repeated injections of estradiol or estrone. The zone is apparently derived from the reticulo-endothelial system and is probably an evidence of antitoxic activity rather than a specific response to sex hormones.—J. C. D.

Action of estrin on the X zone and on the cortex of mice (Action de l'oestrine sur la zone X et sur le cortex de la surrenale chez la souris). Lacassagne, A., and A. Raynaud, Compt. rend. Soc. de biol. **124**:1186. 1937.

Mice were given repeated injections of estrin, in most cases starting a few days after birth. They were examined at various stages until the X zone had disappeared. The involution of the X zone was not modified in either sex. If the injections were sufficiently prolonged, there was a reduction in the size of the true cortex.—J. C. D.

Chemical studies of the suprarenal cortex. III. The structures of compounds A., B. and H. Mason, H. L., W. M. Hoehn, B. F. McKenzie and E. C. Kendall, J. Biol. Chem. **120**:719. 1937.

Various compounds designated *A, B* and *H* isolated from adrenal glands by fractional extraction with benzine, isopropyl alcohol and ethyl alcohol were studied in detail. They were found to be C_{21} steroids with α-Ketol side chains assigned to C_{17}. Compounds A and B have an α, β unsaturated ketone group which was assigned to the same position as in progesterone. In compound H the double bond was absent and an alcohol group replaced the ketone. In compounds A and H there was an inert ketone group at position C_{11}, which was occupied by an alcohol group in Compound B. This position was chosen because of the close correspondence of the properties of the ketone group to those of an inert ketone group at C_{11} in sarmentogenone. Compounds A and B had cortin-like physiologic activity, and Compound B was identical with the corticosterone of Reichstein. The α, β unsaturated ketone group was related to physiologic activity.—J. M. L.

The adrenal cortical syndrome. Meyler, L., and M. Hommes, Acta med. Scandinav. **93**:253. 1937.

Two cases of hirsutism in young girls were described which presented all the clinical characteristics of the adrenocortical syndrome, with obesity, menstrual disturbances, hypertricho-

ses and virilism. Treatment with ovarian extract (menoformon) in dosages up to 30,000 U was followed by a return to normal appearance in both cases. The difficulty of differentiating Cushing's syndrome (basophilic adenoma) from adreno-cortical hyperfunction from other causes was discussed in detail.—J. M. L.

Diathermy and the secretion of adrenalin (Diathermie et adrenalinosecretion). Michez, J., Compt. rend. Soc. de biol. **124**:1006. 1937.

Blood from the adrenal vein of dogs under chloral was tested for its inhibitory action on rabbit's intestine. The increased adrenin output following local application of the electrodes is a direct effect on the adrenal, for it occurs after the glands are denervated. General diathermy with the electrodes on the limbs does not cause an increase in adrenin output.—J. C. D.

Does hydrocyanic acid induce an epinephrine discharge? (Ruft Blausäure eine Adrenalinausschüttung hervor?). Møller, K. O., and K. Stefansson, Skandinav. Arch. f. Physiol. **76**:115. 1937.

The injection of 1 to 2 mg. of NaCN per kg. in the rabbit induces an hyperglycemia which lasts for 2 to 3 hours. The effect is not elicited after adrenalectomy.—A. G.

Ascorbic acid in adrenals after death (Action de la cadavérisation sur la teneur des surrénales en acide ascorbique). Mouriquand, G., and P. Viennois, Compt. rend. Soc. de biol. **125**:289. 1937.

The adrenals of guinea pigs after death showed a steadily decreasing content of ascorbic acid. The average loss in 3 groups of 3 guinea pigs each was 25%, 38%, 58%, after 24, 48 and 76 hours, respectively.—E. Cutuly.

The innervation of the suprarenal glands. Swinyard, C. A., Anat. Rec. **68**:417. 1937.

Section of appropriate thoracic nerves in the cat brought about degeneration in 50 to 70% of the nerves in the suprarenal. The lower thoracic nerves were found to be the most important source of fibers. There was no evidence of afferent fibers in the glands. Postganglionic fibers were traced into the suprarenal glands from terminal ganglia located on the course of the nerves both in connective tissue around the glands and in the capsule. The recent work of Hollingshead was corroborated with respect to the presence of preganglionic fibers, general distribution of nerve fibers to the medulla and absence of cortical innervation. Human suprarenal glands were studied in serial section, stained with the Davenport silver method, Bielschowsky technic and the Pal-Weigert method. The Bielschowsky technic was shown to be unreliable, it being impossible to distinguish between nerve fibers and reticular fibers. The Davenport method completely suppressed the reticular tissue and yet the finest nerve fibers were clearly shown. No evidence of a cortical innervation of the human suprarenal was shown in sections stained with the Davenport silver method. The medulla, on the other hand, is richly supplied with nerves. The abundant cortical innervation described by some was not found. This difference is interpreted to being due to use of a technic which does not distinguish between reticular fibers and nerve fibres. About one-half of the nerve fibers approaching the human suprarenals are myelinated.—Authors' Abst., Wistar Bib. Service.

New studies on potassium in adrenal insufficiency (Nouvelles recherches sur le potassium dans l'insuffisance surrénale). Urechia, C. I., G. Benetato and Mme. Retezeanu, Compt. rend. Soc. de biol. **125**:191. 1937.

Potassium determinations were made on the organs of normal and adrenalectomized (12) tadpoles. Adrenalectomy caused a reduction of K in organs and muscle tissue and a retention of K in the blood.—E. Cutuly.

Modifications in testicular structure in adrenalectomized cats (Contribution à l'étude des modifications de la structure du testicule chez les chats surrénalectomisés). Waitz, R., Compt. rend. Soc. de biol. **125**:140. 1937.

Complete atrophy of the seminiferous epithelium of the testicles was observed in 4 cats which had survived adrenalectomy for 34 to 73 days with little or no adrenal cortical hormone therapy; the interstitial cells of the testes were but slightly modified. Daily administration of 3 cc. of cortical extract (cortin) for 34 days to one cat almost completely prevented tubular damage in a biopsied testis; the second testis of this cat which finally died of adrenal cortical insufficiency revealed tubular atrophy. Cats which survived double adrenalectomy for 12-16 days, and

which had been treated with varying doses of cortical hormone, had testes whose tubular epithelium showed different amounts of degeneration ranging from negligible to profound. The dose of cortical hormone necessary to prevent tubular atrophy was approximately that required to maintain an animal in good health. It was suggested that an assay of the adrenal cortex hormone might be based on these findings.—E. Cutuly.

Testicular structure and nutritional disturbances in adrenalectomized cats (Structure testiculaire et troubles nutritifs chez les chats surrénalectomisés). Waitz, R., Compt. rend. Soc. de biol. **125**:142. 1937.

Inanition in cats, resulting in marked losses in body weight, did not cause damage to the seminiferous epithelium of the testes. Adrenalectomized cats receiving adequate adrenal cortical hormone therapy, although undergoing great loss in body weight, possessed normal tubular tissue. While followed by a proportionally small drop in body weight, suspension of cortical hormone therapy in an adrenalectomized cat resulted in tubular atrophy. The interstitial cells in the testes of adrenalectomized cats with atrophic germinal epithelium remained normal, histologically and physiologically (maintenance of prostatic epithelium). The question is raised whether the essentiality of the adrenal cortex to normal seminiferous epithelium is direct or indirect.—E. Cutuly.

ENDOCRINE GENERAL

Quantitative effects of theelin on body growth and endocrine glands of young albino rats. Freudenberger, C. B., and F. W. Clausen, Anat. Rec. **69**:171. 1937.

Two groups of immature female Wistar albino rats were injected with 200 I.U. (60 R.U.) of theelin in oil daily, beginning at the 21st day of age. One group was injected for a period of 5 days and autopsied, the other group for a period of 10 days and then sacrificed. Both groups had litter-mate controls which were injected with equal amounts of olive oil. The hypophysis was significantly enlarged in the 5- and 10-day groups. The thyroid gland was enlarged in both groups, but significantly so in the 10-day group only. The ovaries were also enlarged in both groups, but significantly so in the 5-day group only. The thymus gland was significantly decreased in weight in the 5- and 10-day groups. The body weight was significantly decreased after 10 days, the change being first significant after 6 days. The tail length was significantly smaller after 10 days of injection. The head weight, suprarenal glands, and body length showed no significant changes in either group.—Authors' Abst., Wistar Bib. Service.

GONADS

An experimental study of plumage in Reeves pheasants. Danforth, C. H., J. Exper. Zoöl. **77**:1. 1937.

In the Reeves pheasant there are well-marked and consistent differences in the responses of feather follicles of male and female skin to female hormones at all concentrations within the normal range. Specific responses to a testicular hormone have not been detected, but developing feathers of the two sexes do not react in the same way in the presence of the total endocrine environment of the male. Feather producing tissues of each sex are especially responsive to their own appropriate humoral environment and, from the point of view of normal development, rather refractory to that of the opposite sex.—Author's Abst., Wistar Bib. Service.

Biology of the human vagina in pregnancy. Davis, M. E., and S. A. Pearl, Am. J. Obst. & Gynec. **35**:77. 1938.

Numerous microphotographs illustrate the presentation of a study of 30 pregnant women by vaginal biopsies, correlated with stages of pregnancy and puerperium. The vaginal mucosa responds as it does in infancy, childhood, maturity, and the climacteric to an increase and decrease of estrin, with consequent proliferation of more numerous cell layers, presence of liberal amounts of glycogen, and the lactic acid forming flora whenever estrin production is increased. The significance of pathology and therapy is presented briefly.—E. L. S.

Menstruation and its disorders. A critical review of the literature from 1933 to 1936 inclusive. Ehrenfest, H., Am. J. Obst. & Gynec. **34**:1051. 1937.

This is a well-organized review, concluded from the Sept. and Oct. 1937 issues of the same journal, ably documented with 394 references, which the author has brought together with a conservative but fair point of view. The arrangement is by conventional gynecological diagnostic terms. Organotherapy is included in this latter third.—E. L. S.

A summary of data for the effects of ovariectomy on body growth and organ weights of the young albino rat. Freudenberger, C. B., and E. I. Hashimoto, Am. J. Anat. **1**:93. 1937.

Twenty-five Wistar albino rats were bilaterally ovariectomized at an average age of 26 days and necropsied at 70 days. Control and spayed body and organ measurements were made. Biometrical methods were applied in the analysis of the data; and these results, in turn, were compared with results from two groups of rats autopsied at 90 and 184 days, respectively. In all the spayed animals the weights of the body, head, integument, humerus, thymus and stomach were increased; but only in the younger groups were the lengths of the body, tail, femur, humerus, and the weights of the femur, brain, hypophysis, heart, lungs, spleen, submaxillary glands, alimentary group, intestines, liver and kidneys increased. Questionable alteration as a result of ovariectomy was found in the weights of the spinal cord at 70 days; and in tail length, and the weights of the heart and lungs at 184 days. The thyroid weight was questionably increased at 70 days, significantly heavier at 90 days, and unchanged at 184 days. The uterus was significantly smaller in all spayed animals. No changes occurred in the weights of the eyeballs. The suprarenal glands were decreased in weight at 90 and 184 days, but unchanged at 70 days.—Authors' Abst. Wistar Bib. Service.

Hormone sensitization test for pregnancy. Greene, G. B., South. M. J. **30**:727. 1937.

In 57 cases, of which 20 were pregnant, the test was accurate.—J. C. D.

The quantitative determination of estrogenic substances in normal female urine during the menstrual cycle. Gustavson, R. G., L. W. Mason, E. E. Hays, T. R. Wood and F. E. D'Amour, Am. J. Obst. & Gynec. **35**:115. 1938.

The technic is acid hydrolysis of 24-hour urine samples, followed by continuous chloroform extraction, purification leading to solution in 95% alcohol, and final administration of an aqueous emulsion of the residue after evaporating alcohol. A few complete menstrual cycles are studied, some without the hydrolysis of estrin esters. The authors are impressed with the presence of two peaks of excretion, followed by sharp drops, but these peaks do not come at the same time in all cycles. The occurrence of the early peak well before the assumed ovulation time of about 14 days previous to flow leads to speculation as to the times when the follicle is most active in producing estrin.—E. L. S.

Menstruation inhibiting action of testosterone. Hartman, C., Proc. Soc. Exper. Biol. & Med. **37**:87. 1937.

In a monkey with very regular periods (24-28 days) the daily injection of 10 mg. of testosterone proportionate from the 20th day of the cycle until 12 days later resulted in a period of 38 days. Similar injections into a monkey immediately following castration prevented bleeding for the 12 days of observation. Finally, this same dose of testosterone started on the day of cessation of estrin treatment prevented bleeding from the estrin withdrawal for the entire period of observation, which in one case was 63 days. In these same monkeys control experiments had shown the estrin dosage employed to provoke bleeding upon withdrawal. Although there was some mammary proliferation, there was no pregravid transformation of the endometrium by the testosterone. The sexual skin remained as red during these testosterone injections as during the estrin injections.—M. H. F.

Prolongation of pregnancy in the rabbit by the injection of progesterone. Heckel, G. P., and W. M. Allen, Am. J. Obst. & Gynec. **35**:131. 1938.

Rabbits are treated with natural and synthetic progesterone, known to be estrin free, with doses of 0.25 to 2.0 mg. beginning on the 20th to 28th days of the gestation (normal duration 32 days), and continuing daily until delivery or autopsy. Significant prolongation of pregnancy occurred when the doses were 1.5 mg. or over, even beginning as late as the 28th day. Fetuses became post-mature, but did not survive longer than the 35th day at most. When parturition did occur, it was often abnormal and prolonged.—E. L. S.

Contribution to the development of an improved method of assaying estrogenic substances. Hemmingsen, A. M., and E. T. Nielsen, Skandinav. Arch. f. Physiol. **76**:213. 1937.

By determining the varying composition of the vaginal smear, it is possible to assay water soluble estrogens in the spayed rat within 62 hours after the first injection. Solutions containing from 0.2 to 6.0 R.U. can be accurately assayed.—A. G.

The structure of the secreting and retrogressing mammary gland in the guinea pig. Hesselberg, Cora, and L. Loeb, Anat. Rec. **68**:103. 1937.

Microscopic changes which take place in the mammary gland of the guinea pig during lactation and the subsequent stage of retrogression are described. Two kinds of acini are found in the lactating gland and combinations of secreting and retrogressing acini appear in both lactating and retrogressing glands, but in different proportions. There may take place a complete granular degeneration of retrogressing acinar cells. The increase in connective tissue during retrogression is not only relative to the amount of mammary gland tissue, but an actual new formation of connective tissue may occur. The retrogressive changes are the same in a mammary gland of one side ligated during lactation and in the gland during the stage of normal retrogression.—Authors' Abst., Wistar Bib. Service.

The management of tumor of the testicle. Hinman, F., and T. O. Powell, J. A. M. A. **118**: 188. 1937.

Usually the general practitioner of medicine, not the specialist, first sees the patient with a neoplasm of the testicle. Although uncommon, such tumors are not extremely rare. Most of them are very malignant, and it is only by early diagnosis and proper treatment that any patients are cured. The authors discuss what would seem to be the logical management of the patient in the light of recent knowledge. Two illustrative cases of unusual interest are reported. An analysis of 58 cases of tumor of the testicle, in all of which hormone tests were made, forms the basis for the outline of management. The majority of the 58 patients have been observed for 3 years or longer. The steps to be followed in the logical management of tumor of the testis are: clinical history; physical examination; hormone test of the urine; orchidectomy; histologic-hormone classification of the tumor; prognosis and subsequent treatment.—Abst., A. M. A. Service.

The effects of oestrin-progestin combination on the endometrium, vagina and sexual skin of monkeys. Hisaw, F. L., R. O. Greep and H. L. Fevold, Am. J. Anat. **61**:483. 1937.

Castrated, adolescent, Macacus rhesus monkeys were given daily injections of estrin and progestin, combined in varying amounts, for 10 to 59 days and the effects on the uterus, cervix and sexual skin were studied. The uteri were much larger than those of animals that received the same dosages of estrin or progestin alone for comparable periods. Fully developed premenstrual endometra were produced and the glands showed different degrees of secretory exhaustion depending upon the length of the treatment. Uteri traumatized by biopsy or by placing threads through them between the eighth and tenth days after beginning the combination treatment developed endometrial proliferations comparable to normal implantation sites. Such proliferations apparently originated from the epithelium of the uterine lumen and the glands. Giant cells and multinucleated cell masses were common and they underwent vacuolation and disappeared when treatment was continued longer than 15 days. The cornified condition of the vaginal epithelium decreased when progestin was given simultaneously with estrin and disappeared when the combined treatment was prolonged. Also, the edema of the sexual skin was lost when progestin was added to an estrin treatment. Estrin and progestin had a greater effect on the uterus when injected simultaneously than when given separately. Progestin decreased or inhibited the action of estrin on the cervical glands, vaginal mucosa and sexual skin.—Authors' Abst., Wistar Bib. Service.

Virilism. A case report and discussion. Howard, J. E., and M. R. Whitehill, Internat. Clin. **4**:(series 47)50. 1937.

This is a useful discussion of the subject of virilism in general, and of a case in particular. The patient described was an 18-year-old girl who presented the clinical picture of virilism, signs of which began to appear at the age of 9. A tumor of the left suprarenal was found and removed. Pathologically the tumor was a cortical adenoma. In the 7 months since operation, hirsutism has decreased to some extent, menstruation has begun and become regular, the breasts have developed, and the uterus has enlarged.—I. B.

The inactivation of estrogen by the liver. Observations on the fate of estrogen in heart-lung and heart-lung-liver perfusion systems. Israel, S. L., D. R. Meranze and C. G. Johnston, Am. J. M. Sc. **194**:835. 1937.

The comparative rates of disappearance of known amounts of estrogen from standing dog blood and from dog blood circulating in heart-lung and heart-lung-liver perfusion preparations

were determined (3 experiments). The estrogens were assayed by the spayed-rat vaginal smear method. These experiments show that estrogen is not inactivated by dog blood in vitro or by the circulation in a heart-lung perfusion system. It is rapidly inactivated by circulation in a heart-lung-liver perfusion system. From these observations, it is concluded that the liver, in some manner, either destroys or alters circulating estrogen. The possible operation of a liver estrogenase is suggested.—R. G. H.

Functional uterine bleeding with special reference to that associated with secretory bleeding. Jones, H. W., Am. J. Obst. & Gynec. **35**:64. 1938.

After a lengthy discussion of the previous reports on the types of endometrium found in women with menorrhagia, 83 consecutive cases with this complaint are reported, with 14% having secretory endometrium. Another series of 41 cases, in which secretory endometrium was found associated with menorrhagia, are studied in detail. The complaint is usually simple excessive flow, but in a few patients the flow was very long (up to 34 days), or associated with corpus luteum cyst. Half the patients in this group of 41 had normal menstrual intervals, one-fourth had shortened intervals, and one-fourth had irregular intervals. Three-fourths of the group are below 40 years of age.—E. L. S.

Absorption and excretion of oestrone by the human organism. Kemp, T., K. Pedersen-Bjergaard, Lancet **233**:842. 1937.

In the administration of estrone to men or castrate women there is a recovery of 3-12% in the urine. If given by mouth the proportion of estrone excreted is proportional to the dose administered; therefore, in the opinion of the authors it has a low specific biologic action, for it is rapidly absorbed and found in high concentration in the blood. To be effective orally it must be given in divided doses.—L. B. S.

Modifications of the clitoris in mice under the influence of testosterone from birth (Modifications du clitoris de la souris sous l'influence de la testostérone agissant dès la naissance). Lacassagne, A., and A. Raynaud, Compt. rend. Soc. de biol. **125**:351. 1937.

Testosterone acetate was administered to a litter of mice once a week, the injections being begun on the day of each animal's birth. One animal was killed each week, the final autopsy occurring after 6 weeks. The penis in the males did not show any apparent anomaly. In females the clitoris was hypertrophied. One clitoris showed a dorsal median cleavage furrow lined with scales, a ventral furrow and lateral masses of erectile tissue covered by a congested mucous membrane; in the central part was found a true os penis. Gross and histological details and a discussion of the findings are presented.—E. Cutuly.

Comparison and rate of testicular degeneration in rats after cryptorchidism and hypophysectomy. Leonard, S. L., and J. B. Hamilton, Anat. Rec. **68**:497. 1937.

One testis in each of a group of normal and hypophysectomized male rats was confined to the abdominal cavity, and the other testis was retained in the scrotum by ligatures. The percentage difference in weight between the abdominal and scrotal testes was plotted against varying periods of time following the operative procedures. In the normal rats, the maximum percentage difference in testis weight was approximately 60% in ten days, after which no further decrease in the cryptorchid testes was noted up to 60 days. In the hypophysectomized rats, the maximum percentage difference between the abdominal and scrotal testes was 39% at 6 days, after which the percentage difference decreased to approximately nil at 20 days. The degree of testicular degeneration in the cryptorchid testes of the hypophysectomized rats at 10 days was equal to the scrotal testes of rats autopsied at 20 days. Microscopic examination of the gonads confirmed the gross changes observed. Confirmatory evidence was presented that cryptorchidism which normally follows hypophysectomy does not aid in the degenerative changes which follow removal of the hypophysis.—Authors' Abst., Wistar Bib. Service.

The question of the endocrine origin of prostatic hypertrophy (Au sujet de l'origine endocrine de l'hypertrophie prostatique). Le Roy des Barres, A., Bull. Acad. de méd., Paris **117**:367. 1937.

Studies in Tonkin revealed a complete absence of prostatic hypertrophy among the natives, whereas the white population showed the average incidence. It is concluded that the reduction of male sex hormone with age is not the exclusive factor that determines the formation of prostatic adenoma.—A. E. Meyer.

Endocrine treatment of vaginitis of children and of women after the menopause. Lewis, R. M., and Eleanor L. Adler, J.A.M.A. 109:1873. 1937.

By treating a small series of children with gonorrheal vaginitis with quite large amounts of ethylene glycol amniotin hypodermically the authors cured the disease in an average of 24.2 days. These results were confirmed by some clinics, although others did not meet with the same therapeutic success. Treatment solely with vaginal suppositories of amniotin as described by TeLinde and Brawner has proved vastly more simple and effective than the use of hypodermic or oral preparations. The authors summarize their results in 82 cases of gonorrhea vaginitis in children treated only with amniotin suppositories in the Children's Medical Service of Bellevue Hospital, New York. The recent results of other clinics, furnished them in personal communications, are also included. To be specific, this form of treatment consists in the insertion of a gelatin capsule containing 75 R.U. of amniotin, or one-half of an amniotin suppository, into the vagina each night at bedtime. (One half of a suppository contains 1,000 international units of estrogenic substance.) No douches are used, but at first the external genitalia may require cleansing if the discharge is profuse. Of the 82 patients alone in the Children's Medical Service at Bellevue 2 were not cured. The remaining 80 are apparently well. Twenty-nine have been cured for over one year, 21 for over 6 months and the other 30 have been apparently well (negative smears, and the like) for from one to six months. One of the two uncured cases was puzzling because there was no physiologic response even after the administration of large amounts of the substance. Until recently the treatment of senile or postmenopausal vaginitis has been most unsatisfactory. In the authors' experience, results have been good when the condition treated with 100 R.U. of amniotin hypodermically was a typical senile vaginitis. Vulvar leukoplakia has not been benefited. They have had two cases, one after removal of the ovaries and one following intra-uterine irradiation, in which the shrunken vagina became so dry and sensitive that intercourse was impossible. In both instances treatment with amniotin was effective in relieving the situation. It is probable that, as well as building up the vaginal mucosa, secretion from the cervix and Bartholin's glands was restored.—Abst., A. M. A. Service.

A pharmacological ejaculation test for bio-assay of male sex hormone. Loewe, S., Proc. Soc. Exper. Biol. & Med. 37:483. 1937.

Several androgens were assayed on castrate mice by the determination of the dose necessary to elicit ejaculation in 50% of the animals when they were properly injected with a pernoston-yohimbine combination. For testosterone the unit was found to be about 40 gamma, and the ratios of the testosterone potency to that of other androgens was found to be similar to the ratio found for other mammalian tests, and thus much different from the ratios obtained with bird tests. With this test, one can tell not only the incidence of response in the group of animals but also the duration of the effect in each animal by repeated tests every 2 to 4 days.—M. H. F.

The hormonal basis for Witches' Milk. Lyons, W. R., Proc. Soc. Exper. Biol. & Med. 37: 207. 1937.

The urine of 4 new-born babies was extracted and tested for mammotropin content by the local crop sac method. Positive responses were obtained from each of the urines, the greatest hormone content being found in a lactating boy. Despite the hormonal analysis, 2 of the 4 babies showed no breast growth or milk secretion during the week of observation.—M. H. F.

Testis hormone in the urine in normal and pathological cases. McCahey, J. F., L. P. Hansen and D. Soloway, J. Urol. 38:397. 1937.

In 61 cases the urine was assayed for testis hormone by measuring its power to produce growth in the comb of a capon. The yield of testis hormone from the urine of boys, especially those below the age of puberty, was so small that these methods seem of no value in the study of hypogonadism in such cases. The methods are suitable in cases of hypergonadism. The comb-growth response in the normal men studied was found to lie within certain ranges. Patients with dysfunction of the spermatogenic epithelium were found to excrete testis hormone within the ranges of normal men. High ranges of testis hormone excretion were not found in cases of prostatism. Much less testis hormone was found in the urine of patients of the nonobese type of hypopituitarism than in the other types of this condition which were studied. Cases with the physical finding of pads of girdle fat were not all associated with low testis hormone excretion. The significance of this condition as indicating lowered activity of the gonadotropic cells of the anterior pituitary is questioned. High urinary contents of testis hormone were found in 3 cases of hirsutism and one case of amenorrhea.—J. C. D.

The effects of various gonadotropic substances and thyroxine on the ovaries of horned lizards (Phrynosoma Cornutum). Mellish, C. H., and R. K. Meyer, Anat. Rec. **69**:179. 1937.

Groups of sexually inactive female horned lizards were kept for 9 days under conditions of continuous artificial lighting, a temperature of 32°C. and an oversupply of food. When they were injected with follicle-stimulating, luteinizing and gonadotropic-inhibiting fractions of APE and with the serum of pregnant mares, a large increase in ovarian weight was produced as a result of increased yolk deposition. Animals kept under the same conditions but injected with thyroxine had ovaries which were atrophic in appearance and which weighed less than those of controls. Other lizards were kept in hibernation at a temperature of 5°C. Their ovaries failed to respond to treatment with an unfractionated APE—Authors' Abst., Wistar Bib. Service.

Effect of adrenocorticotropic hormone on the sexual development of spayed rats. Moon, H. D., Proc. Soc. Exper. Biol. & Med. **37**:36. 1937.

The daily injection of adrenocorticotropic extract into female rats spayed at 22-23 days of age led to vaginal opening 16 to 24 days later. Uninjected spayed controls did not show vaginal opening during this period of observation.—M. H. F.

Growth hormone and creatinuria (Hormone somatrope et créatinurie). Nitzescu, I. I. and I. Gontzea, Compt. rend. Soc. de biol. **125**:291. 1937.

Administration of growth hormone to human subjects presenting symptoms of infantilism, hypogonadism, nanism, etc., augmented their creatinuria, decreased their tolerance to exogenous creatine, and seemed to inhibit the depressing effects of male hormone on creatinuria. Administered to normal adult individuals, who do not excrete creatine, growth hormone did not induce any creatinuria. It was suggested that the creatinuria of childhood may be due to a high level of growth hormone in the body.—E. Cutuly.

Source of androgenic and oestrogenic substances of the urine. Parkes, A. S., Lancet **233**:902. 1937.

The author considers that quantitative estimation of gross androgenic or estrogenic activity of the urine does not provide information as to the functional state of the gonads. Further progress can be made by considering *a*), androgen/estrogen ratio as suggested by Koch, *b*), estimating various androgens or estrogens, *c*), isolation of new substances in the urine which may be of diagnostic value, regardless of the fact that they may not have biologic activity.—L. B. S.

The growth, maturation and atresia of ovarian eggs in the rabbit. Pincus, G., and E. V. Enzman, J. Morphol. **61**:351. 1937.

A study is made of the development of ovarian ova in mature rabbit does before and during various stages of pregnancy and after the injection of thyroid and anterior-pituitary-like hormones. No appreciable ovogenesis during sexual maturity was found. Nine types of ovarian follicle are distinguished according to size and degree of development. Full ovum size is reached an pre-antral follicles, and is marked by the formation of a dictyate nucleus and slight reduction in nuclear size. Full follicle size is attained much later. The nature of follicle migration in the course of development is described. Ovum migration within the follicle is found to be a consequence of the nature of the egg's attachments to the follicular epithelium. The least atresia is found in young oocytes (10%), the larger follicles showing about 60% atretic at all stages of the reproductive cycle. The hormone preparations administered affect only the largest follicles and their contained ova.—Author's Abst., Wistar Bib. Service.

Endocrine treatment of menopausal phenomena. Pratt, J. P., and W. L. Thomas, J. A. M. A. **109**:1875. 1937.

The authors observed 200 consecutive menopausal cases over a period of several months. Only 100 of the subjects returned often enough to justify tabulation. The 100 cases is a small number from which to draw conclusions but it is sufficient, however, to establish a trend. The symptoms attributed to the menopause are so diverse that it seems unreasonable to consider that all of them are due to ovarian failure alone. The average age of women in the series at the time of observation was 45.6 years. The average age at the onset of the symptoms was 43.5 years. Eleven of the patients had an artificial menopause. The average time that elapsed between the operation and the onset of the first symptoms of the artificial menopause was ten weeks. In general, the symptoms of the artificial menopause were more severe than were those of the natural menopause. The materials used for the relief of the menopausal symptoms were capsules

containing theelol, phenobarbital or lactose, compressed tablets containing emmenin or lactose (but identical in appearance) and ampules of oil alone or theelin in oil. From 64.2 to 85.7 per cent of the patients who received drugs or placebos (but who did not know which) reported complete or partial relief, all the twenty-two patients who knew they were being given phenobarbital were either partially or completely relieved and eight of the twelve who were aware that they were given a bromide mixture experienced partial or complete relief from symptoms.—Abst., A. M. A. Service.

The effect of progestin on the growth response of the uterus to chronic distention. Reynolds, S. R. M., and W. M. Allen, Anat. Rec. **68**:481. 1937.

The effects of progestin upon the uterus of the rabbit have been determined when the uterus is subjected to graded degrees of distention by means of paraffin pellets of known sizes. The amount of growth in the distended regions is estimated by comparison of the cross sectional areas of the distention sites with the cross sectional areas of undistended areas of the same tissues. It is found in sexually mature rabbits that uterine growth will result from chronic uterine distention in the presence of 6.6 Rb.U. of progestin, but the amount of distention necessary to elicit a given amount of growth exceeds that which is necessary to produce the same amount of growth in untreated, ovariectomized rabbits. In immature estrin injected rabbits treated with progestin, no significant amount of growth is elicited by chronic uterine distention. Endometrial proliferation is, however, marked in all instances. Comparison of these effects with those obtained in estrin treated rabbits is made, and some of the probable factors concerned in these effects are discussed.—Authors' Abst., Wistar Bib. Service.

Changes in electrical potential during oestrous cycle of the rat. III. Vaginal oestrus. Rogers, P. V., Proc. Soc. Exper. Biol. & Med. **37**:212, 1937.

The production of vaginal cornification by intravaginal application of estrin did not result in the same electrical potential in the vagina which is found during spontaneous estrus, or in estrus induced in spayed animals by the subcutaneous injection of estrin.—M. H. F.

Comparative action of testosterone and its esters on the secondary sex characteristics (Etude comparative de l'action de la testostérone et de certain de ses esters sur les caracteres sexuels secondaires). Roussel, G., P. Gley and G. Paulin, Bull. acad. méd. **117**:197. 1937.

Capons to be used for assay of sex hormones are oversensitive if the castration has been performed too recently. Only older birds should be used. The free testosterone is extremely weak as compared with its esters. In the capon, the formiate is the most efficacious, followed by the acetate and propionate, whereas the latter is most active in the castrated rat. Prolonged treatment with a constant dose produces in the capon a stationary development of the comb. Further growth is obtained only by increase of the dose.—A. E. Meyer.

The relation of cyclic changes in human vaginal smears to body temperatures and metabolic rates. Rubenstein, B. B., Am. J. Physiol. **119**:635. 1937.

Vaginal smears, body temperatures, and basal metabolism were studied in 16 normal women. The lowest body temperature occurred at the time of the ovulation smear, the highest occurred at the time of the premenstrual smear. No significant variations were found in basal metabolism, although the metabolic rates were slightly lower during the pre-ovulation phase than during the premenstrual phase. Resting pulse rate and blood pressure showed no correlation with the vaginal smears.—F. A. Hitchcock.

Hormone excretion in cases of prostatic hypertrophy. Rusch, H. P., and P. R. Kundert, J. Urol. **38**:316. 1937.

Forty-eight hour specimens of urine from 16 patients with prostatic hypertrophy were tested for estrogenic and androgenic hormones. Bio-assay on rats and mice showed an average output of 21.5 U of estrogenic hormone and 21.8 U of androgenic hormone. For 12 normal males, the figures were 22.1 and 55.1. In the group studied, therefore, there was a decrease in the output of the androgenic hormone but no material change in that of the estrogenic.—J. C. D.

Effect of testosterone propionate upon gonadotropic hormone excretion and vaginal smears of human female castrate. Salmon, Udall, J., Proc. Soc. Exper. Biol. & Med. **37**:488. 1937.

A surgical castrate was given a total of 815 mg. of testosterone propionate over a period of 27 days. After 465 mg. had been administered (about the 20th day of treatment) the gonado-

tropic hormone began to disappear from the urine. At about the same time the vaginal smear assumed a 'positive' character, and the patient reported relief from her symptoms (hot flashes and headaches, etc.). About 20 days after cessation of the treatment, the gonadotropic hormone again appeared in the urine, the smears became more 'negative,' and the symptoms again were noted. It is concluded that in the human castrate testosterone can produce the same effect as estrin on gonadotropic excretion and on the vaginal smear.—M. H. F.

Effect of testosterone injections upon the course of pregnancy in unoperated and castrated rats. Scipiades, E., Proc. Soc. Exper. Biol. & Med. 37:242. 1937.

Injections of testosterone proprionate in doses from 1 to 5 mg. daily prolonged pregnancy in normal female rats, and prevented abortion in rats castrated during the last third of pregnancy. The injections, however, seemed to exert a deleterious effect on the fetuses, in so far as most of the young born after the injections were stillborn.—M. H. F.

Accentuation of the growth effect of theelin on genital tissues of the ovariectomized mouse by arrest of mitosis with colchicine. Smith, G. M., and W. U. Gardner, Am. J. Anat. 61:321. 1937.

By the use of colchicine which arrests dividing cells in metaphase, the evidence of growth of genital tissues (mitotic division) resulting from theelin stimulation can be greatly accentuated. This method shows clearly the beginning, sequence and extent of growth activity in the tissues. A single injection of theelin in oil started growth prior to 9½ hours and produced a maximum growth activity at 37 hours. This had declined slightly by 48 hours. The dividing cells accumulated as a colchicine effect indicated clearly that the growth response to theelin involves especially the epithelial tissues of the genital organs, at least in the early stages of the recation in adult ovariectomized animals. Muscular, vascular and connective tissue and sympathic ganglion cells of adults as treated in this experiment did not respond by cell division. The growth response in the vagina may be uneven, sometimes starting first in the lateral walls. The appearance of mitoses in the uterine epithelium was accompanied or preceded by an edema of connective tissue.—Authors' Obst., Wistar Bib. Service.

Synthetic proluton (Ueber synthetisches Proluton). Stemmer, W., Zentralbl. f. Gynäk. 61:2592. 1937.

This is a clinical summary of the treatment of women with varied disorders, by use of 1 to 4 mg. progestin daily. There are no data such as biopsies of the endometrium or other biological evidences of endocrine influence which make decisions about effectiveness of the therapy possible. Conclusions are drawn from subjective relief from nervous symptoms or cessation of bleeding. Therapy is sometimes complicated with other materials, the commercial (German) names being used. The author presents several cases of improvement from the bleeding associated with cystic metropathia, threatened abortion, clotted flows, amenorrhea, and toxemias in the first half of pregnancy. The progestin is said to be effective in stopping lactation.—E. L. S.

Influence of ovariectomy on development and growth of tar tumors (L'influence de la castration ovarienne sur le développement et la croissance des tumeurs du goudron). Tavares, A., and E. Morais, Compt. rend. Soc. de biol. 125:179. 1937.

Both ears of 25 spayed rabbits and of 25 normal female rabbits were painted twice a week with tar for as long as 7 months. Of the 25 ovariectomized rabbits 10 died 5-77 days after institution of treatment without showing tumor formation. Tuberosities were observed in the remaining ovariectomized animals as early as 34 days after treatment, but in most cases they did not appear before the 80th day. On histological examination 62, 151, and 206 days, respectively, after treatment, three of these growths proved to be carcinomata. Of the normal tar-painted rabbits 11 died 3-125 days after treatment without showing any neoplasms. The earliest appearance of tumor in the remaining animals was recorded on the 18th day, and biopsies revealed 5 carcinomata 21, 64, 69, 155 and 158 days, respectively, following the first tar application. It was concluded that ovariectomy inhibited the development of tar tumors.—E. Cutuly.

The value of Kustallow's pregnancy reaction with infusoria (Der Wert der Kustallow'schen Schwangerschaftsreaktion mit Infusionstieren). Ulrich, A., Zentralbl. f. Gynäk. 61:2796. 1937.

Based on 142 specimens examined, the test is held to be non-specific and undependable.—E. L. S.

Inhibition of menstruation and ovulation by means of testosterone propionate. Zuckerman, S., Lancet 233:676. 1937.

Injection of 25 mg. of testosterone proprionate twice weekly into mature female rhesus monkeys stopped the menstrual cycle during the period of injections, inhibiting follicular growth and luteinization.—L. B. S.

HYPOPHYSIS

Pituitary dwarfs: Their growth and treatment. Observations on 12 cases for periods of 1 to 8 years. Bayer, Leona M., and H. Gray, Internat. Clin. 3:15 (Series 47). 1937.

The absolute growth curves of 12 pituitary dwarfs and 1 normal child are presented. Although growth obviously fluctuates, dwarfs in general tend to fall progressively behind in height. The growth records of a normal girl and of the 5 treated pituitary dwarfs are charted, and it is shown that of 7 periods of growth hormone administration 5 coincided with periods of retarded growth, 1 with a period of growth accelerated relative to the subsequent control interval, and 1 with a period of striking acceleration relative to both preceding and subsequent periods. The information to date does not seem extensive enough to warrant further analysis as to what factors were responsible for good and poor results. However, by using the method of relative growth rates, the effect of the administration of growth hormone for periods as short as 3 months can be tentatively assessed. The desirable prerequisite for judgment regarding the feasibility of continuation of treatment is an adequate control period of at least 1 year.—I. B.

The anterior pituitary and its diabetogenic and pancreatotropic (blood sugar decreasing) activity. Elmer, A. W., B. Giedosz and M. Scheps, Acta med. Scandinav. 93:487. 1937.

The acid extract of anterior pituitary prepared by L. Loeb's method caused a prolonged increase in the blood sugar levels of 6 rabbits. Daily injection of aqueous extracts of anterior pituitary powder prepared by the method of Anselmino were made into rats and guinea pigs for 3 to 10 days. No change in the pancreatic islets was found, and no lowering of the blood sugar levels. The anterior pituitary elaborates only a blood-sugar-raising diabetogenic substance, which shows an immediate effect when given intravenously in large doses (1 gm. per kg. body weight). This factor is present in the serum of acromegalic patients who are diabetic. The diabetogenic substance was thought to be linked with thyrotropic hormone.—J. M. L.

The pancreatic stimulating hormone of the anterior lobe of the hypophysis (Sur l'hormone pancreato-stimulante du lobe anterieur de l'hypophyse). Elmer, A. W., B. Giedosz and M. Scheps, Compt. rend. Soc. de biol. 124:823. 1937.

In 2 rabbits, 3 rats, and 3 guinea pigs, there were no changes in the blood sugar value or in the histology of the pancreas for a period of several days after the injection of anterior lobe extract. This contradicts previous authors, who claimed a reduction of blood sugar and the formation of new islet tissue following similar treatment. It throws doubt on the existence of the pancreatic stimulating hormone.—J. C. D.

The development of thyrotropic function in pituitary grafts in the tadpole. Etkin, W., J. Exper. Zoöl. 77:347. 1938.

Pituitary grafts were made into hypophysectomized hosts of the tadpoles of Rana pipiens and R. sylvatica. The hosts were hypophysectomized at the tail bud stage. Grafts were made at this stage and in various stages up to the size normal for metamorphosis. Primordia or differentiated glands were used as grafts, one or several being placed in each host. The functional condition of the graft was ascertained by the progress of the host in metamorphosis. The conclusions arrived at were as follows: 1) Metamorphosis resulting from graft activity was characteristically delayed in respect to time and to the size of the host at which this process began. This was true even if glands from mature individuals were placed in young animals. 2) The metamorphic process in hosts was protracted, the later stages being especially slow in their development. These facts were interpreted as indicating that the pituitary is functionally self-differentiating, being apparently independent of any specific nervous or hormonal stimulation. As a graft it develops its functional activity more slowly and less completely than in its normal site.—Author's Abst., Wistar Bib. Service.

The existence of a hematopoietic hormone in the hypophysis (Sur l'existence d'une hormone hématopoiétique dans l'hypophyse). Flaks, J., I. Himmel and A. Zlotnik, Presse méd. 45:1261. 1937.

The investigation was suggested by the observation of erythremia in Cushing's disease, anemia in Simmonds' disease and the fact that the response to reduced oxygen pressure with

erythropoiesis is absent in hypophysectomized animals. Rats fed on bread and milk with the addition of 2 gm. liver daily showed a variable response in their blood picture, but mostly a decline. Feeding of 2 gm. pituitary substance produced, in some cases, an increase of erythrocytes to values exceeding 10 millions. The hemoglobin value dropped. An aqueous extract (pH 3-4) was deproteinized with sulfosalicylic acid, the excess of the latter was removed, the filtrate neutralized and heated. A single injection of this extract, corresponding to 0.4 gm. pituitary, produced a conspicuous reticulocytosis. (In the summary, however, it is stated that the substance is *not* contained in the deproteinized fraction.) The treatment transforms within 24 hours the yellow bone marrow into the red type, with disappearance of the fat.—A. E. Meyer.

Effects of an anterior pituitary grown preparation on protein metabolism. Gaebler, O. H., and W. H. Price, J. Biol. Chem. **121**:497. 1937.

Large female dogs maintained on a constant diet were injected with 40 cc. of growth, hormone, Antuitrin-G, and the excretion of sulphur, nitrogen, phosphorous, sodium, potassium and chloride determined in 24-hour samples of urine and feces. There was a drop in the N., P. and S. excretion immediately after the injection followed by an increase above that of the control period. The N:S ratio rose sharply in each experimental period. There was a fall in the excretion of inorganic constituents accompanied by a retention of water. The material retained had a high sulphur content which was thought to be associated with glutathione retention, and the fall in nitrogen retention to a diminished urea output.—J. M. L.

The relation of the Golgi material to the secretory process in the basophilic cells of the anterior hypophysis. Gatz, A. J., Anat. Rec. **69**:429. 1937.

There is a definite inter-relationship between the Golgi material and the secretory cycle of the basophilic cell which may be outlined in the following manner: 1) There are osmiophilic granules within the central portion of the Golgi material. 2) Secretion droplets, of varying sizes, surrounded by an osmiophilic girdle within the central portion of the Golgi material. 3) Droplets in the osmiophilic periphery of the Golgi material. 4) Secretion droplets surrounded by an osmiophilic girdle at the periphery of the small forming vacuoles, as well as at the periphery of the large vacuoles in the vacuolated cells. 5) Osmiophilic granules and particles located in the cytosome of the large vacuolated cells. These fragments are apparently the remnants of the osmiophilic girdle which previously had surrounded the secretion droplet. The Golgi material may serve an enzymatic function or act as an intracellular center for chemical synthesis. In performing this function, some of the 'body' of the Golgi material becomes transformed into small osmiophilic girdles containing droplets of secretion. The liberation of the secretion causes the appearance of the osmiophilic fragments which are seen in the vacuolated basophiles.—Authors' Abst., Wistar Bib. Service.

Blood sugar response to intraperitoneal epinephrine injections in normal and hypophysectomized dogs. Heinbecker, P., and T. E. Weichselbaum, Proc. Soc. Exper. Biol. & Med. **37**:527. 1937.

In contrast to the differences reported after the subcutaneous injection of adrenin into normal and hypophysectomized animals, repeated blood sugar determinations on 9 normal and 9 hypophysectomized dogs showed no differences in the response to intraperitoneal injections of adrenin.—M. H. F.

A cytological study of the anterior hypophysis of the guinea pig and a statistical analysis of its cell types. Kirkman, H., Am. J. Anat. **61**:233. 1937.

Hypophyses from fetal, immature, adult and castrate males; from fetal, immature, diestrous, proestrous, estrous, postestrous, pregnant, lactating and spayed females were studied with respect to cell types, cytological features (secretory cycle, nuclei, Golgi apparatus, mitochondria, cytoplasmic granules and fibrillae) and miscellaneous features (weight of gland, colloid, cell arrangement and hematopoiesis). Although basophiles and acidophiles appear at about the same time in fetal glands the former is more precocious in development. The relative number of basophiles decreases in estrum. An increase in the relative number of degranulated basophiles occurs both at the beginning and the end of pregnancy. An apparent stimulation of the activity of acidophiles occurs at the middle and the end of pregnancy and following parturition. During the latter part of pregnancy there is an increase in colloid, persisting until after parturition. Following gonadectomy basophiles increase and acidophiles decrease in size and number; some increase in colloid occurs. Gonadectomy changes are more marked in the female than in the

male. The secretory cycle is characterized by progressive cytoplasmic and nuclear changes. Hematopoiesis is absent in both fetal and adult hypophyses. The paper also includes interpretations of observations, an extensive bibliography and numerous statistical tables, diagrams and photomicrographs.—Authors' Abst., Wistar Bib. Service.

The presence of traces of prolactin in the serum of mares during pregnancy and lactation (Presence de traces of prolactine dans le serum de la jument durant la gravidite et la lactation). Leblond, C. P., Compt. rend. Soc. de biol. **124:**1062. 1937.

Administration of serum from lactating cows and mares relieves failure of lactation. This effect, however, is not due to prolactin because when such serum, as well as that from pregnant mares, was tested for its general and local effect on the crop gland of pigeons, the results showed only traces of prolactin.—J. C. D.

Rapid method for studying prolactin (The lactogenic hormone of the hypophysis) (Methode rapide de recherche de la prolactine) (Hormone lactogene de l'hypophyse). Leblond, C. P., and E. Allen, Compt. rend. Soc. de biol. **124:**1190. 1937.

Prolactin injected into pigeons increases the rate of cell division in the crop gland. If colchicine is used to 'freeze' the cells in metaphase, the gland can be used as a test organ and the results read after 10 hours. Forty U of prolactin and 0.5 mg. of colchicine were injected into a 450-gram bird. On examination, 20% of the crop gland cells were in mitosis.—J. C. D.

The presence of the maternal instinct without hormonal stimulation (Presence d'instinct maternel sans stimulation hormonale). Leblond, C. P., and W. O. Nelson, Compt. rend, Soc. de biol. **124:**1064. 1937.

Mice of both sexes were hypophysectomized before puberty. This eliminated the effect of prolactin or other hypophyseal hormone, which influences the maternal instinct. In these animals there was a definite maternal response when new-born mice were put in the cages. The maternal instinct is, therefore, mediated by both the brain and the hypophysis. The relative importance of these two varies in different kinds of animals.—J. C. D.

Retardation of somatic growth in castrate rats with pituitary extracts. Moon, H. D., Proc. Soc. Exper. Biol. & Med. **37:**34. 1937.

When daily injections of large doses of adrenocorticotropic extract were given to castrate immature rats of both sexes, there was almost complete inhibition of somatic growth and considerable retardation of hair growth on shaved areas. Despite this fact, there was very little inhibition of organ growth so that noticeable protrusion of the abdomen was noted.—M. H. F.

Xanthoma diabeticorum. Nicholson, W. M., Internat. Clin. **4:** (series 47) 71. 1937.

A case of xanthoma diabeticorum (a rare condition) is reported because of its occurrence in a patient who showed certain symptoms of Cushing's syndrome. The patient was a white male, 41 years old whose chief complaint was a generalized skin eruption of 5 weeks' duration. For the past 20 years his weight has remained at 220 lb. The relationship of the symptoms of Cushing's syndrome to the production of hypercholesteremia and xanthoma diabeticorum in this patient is, of course, a matter of speculation, but it does suggest that some substance secreted by the anterior hypophysis acted upon the liver to produce an increase in plasma cholesterol and a disturbance in the ratio of cholesterol to cholesterol esters, the result being a precipitation of cholesterol esters in the tissues to form the xanthic lesions.—I. B.

Cushing syndrome in a mulatto. Page, A. P. M., L. V. Roberts and J. H. Biggart, Lancet **233:**625. 1937.

This is the first vertified case of Cushing's Syndrome in a colored subject. The clinical findings were characteristic and verified at autopsy.—L. B. S.

Serial implantation of anterior lobes of bovine and human pituitary glands. Saxton, J., L. Loeb, Arch. Path. **24:**135. 1937.

Seventeen experiments, in which the anterior lobes of bovine and human pituitary glands were implanted serially into guinea pigs, were carried out. The first group consisted of 11 experiments. In this series, as a result of the implantation, there was induced follicular atresia which became gradually weakened allowing the luteinizing factors to assert themselves. After

the substances responsible for luteinization had become sufficiently extracted, the pure maturation effects manifested themselves. The second group, consisting of 6 experiments, indicated that under certain conditions, when the health and weight of the animal became affected, the atresia producing action may continue to be effective throughout the serial implantations, leading to a degenerative process in the follicles.—L. B. S.

Thyroid stimulating and gonadotropic hormones of the human anterior pituitary gland at different ages and in pregnant and lactating women. Saxton, J., and L. Loeb, Anat. Rec. **69**:261. 1937.

The amount of thyroid-stimulating hormone present in the human anterior pituitary gland is shown, on the whole, to be constant throughout the various conditions in the human life. In contrast with this constancy of the thyroid-stimulating hormone, the gonadotropic hormones undergo marked changes. During childhood the maturation hormone is found in the anterior pituitary gland, whereas luteinizing effects on the guinea pig ovary are exerted by the anterior pituitary gland not at all, or only in a rudimentary form. During adult life both follicular maturation and granulosa and theca interna luteinization are induced by the anterior pituitary glands. The same applies in old age, in men as well as in women past the menopause. During pregnancy following the first few months all gonadotropic activity of the anterior pituitary is lacking, a condition which corresponds to the failure of follicles to mature in the ovaries of pregnant women. Early during the period of lactation, first the maturation effects and soon afterward also the luteinization processes are restored.—Authors' Abst., Wistar Bib. Service.

Effects of the thyrotropic hormone of the anterior pituitary in man. Scowen, E. F., Lancet **233**:799. 1937.

The thyrotropic hormone prepared from the anterior pituitary gland of the pig was administered intramuscularly into the buttocks of 2 normal patients, 3 patients with pituitary insufficiency, and 1 patient with myxoedema. The B.M.R. was taken twice weekly. The B.M.R. was raised in the normal patients and in those with pituitary insufficiency. It was, however, without effect in the patient with myxoedema. The authors justifiably conclude that the thyroid gland of man is under the control of the anterior pituitary gland.—L. B. S.

Influence of cattle anterior pituitary extract on endochondral ossification in young ovariectomized guinea pigs. Silberberg, M., and Ruth Silberberg, Proc. Soc. Exper. Biol. & Med. **37**:446. 1937.

Observations covering 4 weeks were made on endochondral ossification in guinea pigs weighing 140 gm. initially. Ovariectomy led to widening of epiphyseal line with delayed ossification. Injections of pituitary extract led to narrowing of the line with accelerated calcification. Similar injections in ovariectomized animals led to a combination of these effects, summated in various ways from animal to animal. However, in general there was a widening of the line with accelerated calcification, although closure was not effected in these animals, as in the normal animals injected with the extract.—M. H. F.

Hypophysectomy and its effects on male reproductive organs in a wild mammal with annual rut (Citellus). Wells, L. J., and E. T. Gomez, Anat. Rec. **69**:213. 1937.

Ninety-eight male ground squirrels (Citellus tridecemlineatus) were employed. Seventy were hypophysectomized, and 31 of these lived for postoperative periods ranging from 25 days to 5 months. Success of hypophysectomy was determined by a study of serial sections of the sellae. Incomplete hypophysectomy during the bleeding season rendered the reproductive organs atrophic within periods of 25 to 30 days. Incomplete removal of the hypophysis in one sexually-regressed adult prevented the normal renewal of sexual development. Complete hypophysectomy of prepuberal males for periods of 3 to 5 months prevented further development of reproductive organs (4 cases). Incomplete removal of the hypophysis before puberty prevented further sexual development in 12 males (66.6%), but failed to do so in 6 others (33.3%). Daily administration of fresh anterior hypophyses from rats to two male ground squirrels, which were completely hypophysectomized before puberty, resulted in each case in the formation of spermatozoa and a sub-maximal development of accessory organs within a period of 30 days. Since sexual development did occur in some males with only small fragments of hypophysis tissue, it is concluded that the presence of tiny pituitary fragments should not be considered too lightly when interpreting results of experiments in which the mammalian male is the test animal.—Authors' Abst., Wistar Bib. Service.

Permanent experimental diabetes produced by pituitary (anterior lobe) injections. Young, F. G., Lancet 233:373. 1937.

Extract prepared from fresh ox pituitary glands, by a method Schockaert described, was injected intraperitoneally into dogs. The urine sugar was estimated by Benedict, urinary ketones were assayed by Rothera and Gerhardt tests, and the blood sugar by the Hagedorn-Jensen method. As a result of daily administration of this extract two dogs became diabetic, and remained so after the injections were stopped. A third animal became only temporarily diabetic. The permanent diabetes produced in this manner differed from that of depancreatized dogs in that the experimental animals were able to survive without insulin, retaining good vigor and in one case without loss of weight. One animal treated experimentally with insulin required 60 U each day to keep the urine sugar free.—L. B. S.

Morphological changes in the pituitaries of rats resulting from combined thyroidectomy and gonadectomy. Zeckwer, I. T., Am. J. Path. 13:985. 1937.

The pituitaries of 32 male and 30 female white rats, thyroidectomized and gonadectomized at the same time (35 to 40 days of age), were studied. The rats were killed from 44 to 171 days after operation. The glands were fixed in Helly's fluid and stained with Mallory's aniline blue stain. It was found that greater hypertrophy occurs after the double operation than after thyroidectomy alone or after gonadectomy alone. Both thyroidectomy cells and castration cells, which are histologically distinct, appear in the same pituitary. It is held probable that the two cells produce different hormones. After the combined operation acidophiles degranulate and largely disappear, just as after thyroidectomy alone.—Author's summary, condensed.

PANCREAS

Action of normal and diabetic sera on animal liver glycogen in vivo and in vitro. De Wesselow, O. L. V., and W. J. Griffiths, Lancet 233:670. 1937.

The injection of human sera into rats is followed by a reduction in the liver glycogen, and the percentage of reduction is the same whether normal or diabetic sera is used. Human sera accelerates glycogenolysis in rabbits' liver bred in vitro, whereas in the rat no such effect is observed. The author believes it unlikely that this effect is due to amylolytic ferments in the sera.—L. B. S.

Diabetes mellitus treated with protamine zinc insulin. Harrop, Jr., G. A., and R. Whitehill, Internat. Clin. 3:27(Series 47). 1937.

The most serious drawback to the use of protamine zinc insulin in the treatment of some individuals lies in its prolonged and not always predictable action. Care is needed in order to avoid recurrent insulin reactions. Protamine zinc insulin is by no means an innocuous therapeutic agent which can be prescribed with relative disregard of consequences. It is very difficult for patients always to adjust their own insulin dosage in the face of minor variations in their daily routine. For patients who will not submit to a carefully regulated dietary regime the authors feel that the use of regular insulin is usually safer and more satisfactory. The greatest field of usefulness for protamine zinc insulin probably lies in the treatment of mild or moderately severe diabetes. In these cases, requiring 2 or 3 doses of insulin daily, more satisfactory regulation can often be attained by the substitution of a single dose of protamine, or of a dose of protamine combined with regular insulin. In the severe case receiving multiple doses of insulin, the substitution of 1 or 2 doses of protamine for regular insulin at times results in an improvement of the blood sugar level.—I. B.

The basal insulin requirement of depancreatized dogs. Greeley, P. O., Am. J. Physiol. 120:345. 1937.

The basal insulin requirement of depancreatized dogs was determined with constant or hourly injections by the trial and error method. Results reported on 12 dogs give values ranging from 0.0051 to 0.035 U/kg./hr. The requirement of individual dogs was quite constant.—F. A. Hitchcock.

Influence of insulin on amino acid utilisation. Mirsky, I. A., S. Swadesh and J. Ransohoff, Proc. Soc. Exper. Biol. & Med. 37:223. 1937.

In completely eviscerated dogs under amytal anesthesia the intravenous injection of glucose at a constant rate, and a large dose of glycine, results in a rather constant blood level of

amino acid. Into such animals the injection of insulin along with these substances led to a marked fall in the amino acid nitrogen in the blood. The authors interpret these data to indicate increased amino acid utilisation under the influence of insulin.—M. H. F.

The nitrogen-sparing action of glucose in normal, phlorhizinized and depancreatized dogs. Mirsky, I. A., J. D. Heiman and S. Swadesh, Am. J. Physiol. **120**:681. 1937.

Using the rise in the non-protein nitrogen of the blood in bilaterally nephrectomized dogs as a measure of the endogenous nitrogen metabolism, the protein sparing action of glucose given intravenously has been studied on normal, phlorhizinized and depancreatized dogs. The glucose administration was followed in all experiments by a decrease in protein metabolism. The decrease was greatest in the phlorhizinized, and least in the depancreatized animals. The authors interpret these results as lending support to the hypothesis that the metabolic disturbance in pancreatic diabetes is due to an alteration in the balance between hepatic glycogenesis and glycogenolysis.—F. A. Hitchcock.

Pancreotoxin treatment in diabetes mellitus. Sakharoff, G. P., and D. M. Rossiisky, Acta med. Scandinav. **93**:239, 1937.

Serum containing pancreotoxin, produced by immunizing animals with extracts of normal human pancreas, was injected subcutaneously in daily doses of 0.1 to 0.2 cc. into 50 diabetic subjects. There was an improvement in the patients' general state, higher working capacity and a decrease or disappearance of the polydipsia and polyuria. In most cases there was a lowering of the blood sugar levels which in some instances reached normal levels. The urinary sugar showed similar changes. The effect of a single course of 20 to 40 injections may persist for a year or more.—J. M. L.

PARATHYROIDS

Myxedema with menorrhagia and tetany as complications following a partial thyroidectomy. Dunn, C. W., and W. R. Nicholson, Am. J. Obst. & Gynec. **35**:165. 1938.

A woman who 3 months previously had thyroidectomy, discharged with basal rate —15%, returned because of menorrhagia, and was found severely anemic and myxedematous. Four days later, during treatment with thyroid, pregnancy urine concentrate, and bed rest, tetanic seizures occurred. Relief was secured by calcium gluconate intravenously, and maintenance therapy was based on calcium gluconate orally, with viosterol minims 15 daily.—E. L. S.

Hyperplasia of the parathyroid glands secondary to renal insufficiency. Report of a case. Nelson, A. A., Arch. Path. **23**:30. 1937.

A case of hyperplasia of the parathyroid glands associated with polycystic kidneys, in a white woman aged 47, is reported. The parathyroids showed essentially hyperplasia of the chief and oxyphil cells. There were no 'wasserhelle' cells.—L. B. S.

THYROID

Thyroid and testicular growth in the domestic drake (Thyroïde et croissance testiculaire chez le canard domestique). Benoit, J., Compt. rend. Soc. de biol. **125**:459. 1937.

The inhibition of spermatogenic activity and testicular growth which has been observed in thyroidectomized drakes exposed to strong electric light 14 hours a day for 4 to 5 weeks tended to disappear in birds similarly treated for longer periods.—E. Cutuly.

Psychotherapy in treatment of exophthalmic goiter. Bram, I., Internat. Clin. **4**:(series 47)94. 1937.

The presence in our midst of many unsuccessfully treated cases of Graves' disease would seem to indicate that there is a missing link in the therapy of the average case of this malady. This link is psychotherapy. Much of the really good results claimed for surgery, roentgen ray treatment and medical attention practiced by representatives in their fields are assignable to the associated treatment of the individual as a whole. While psychotherapy alone is usually ineffectual, the process of mental adjustment should be regarded as the foundation upon which other more specific measures depend. The sufferer from exophthalmic goiter is a chronically maladapted individual; this can be read into the etiology and the symptomatology, and must receive basic consideration in a broad perspective of treatment. In the majority of patients having received the needful psychotherapeutic attention, the behavior pattern is decidedly modified.

They are calmer than ever before and capable of meeting environmental situations with equanimity. Such a discharged patient sees his environment less through the medium of his emotions and more through the calculations of logic. The former maladjusted individual is now as emotionally stable as are those apparently insusceptible to the disease. Patients treated as a whole, i.e., psychotherapeutically as well as physically, are not prone to relapse. Taught how to eat, how to sleep, how to work, how to play, how to laugh, and even how to think, they enjoy normal health and are capable of meeting life's problems with intelligence.—Author's Abstract.

Hyperthyroidism and personality. Brown, W. T., and E. F. Gildae, Am. J. Psychiat. 94:59. 1937.

A careful study of 15 patients is reported. It appeared that there is a constitutional predisposition to hyperthyroidism, implying a sensitive thyroid gland as indicated by high incidence of nervous irritability in relatives. "The way of experiencing" of persons constitutionally so predisposed involves an extreme feeling of personal insecurity; a strong sense of responsibility; and a tendency to turn the effects of emotional experiences within rather than give them outward expression. Any threat to the security of persons with this constitution and way of experiencing may produce a serious emotional disturbance.—R. G. H.

Anatomic changes produced by thyroid gland feeding and injection of 3,5-diiodotyrosine. Connor, C. L., Arch. Path. 24:315. 1937.

In these experiments the rabbits were divided into two groups. Group *1*, 10 rabbits received 60 mg. of U.S.P. thyroid by mouth each day for 10 months. The animals of this group were emaciated, and had enlargement of the heart with areas of focal necrosis in the myocardium, degeneration of eye muscles similar to that found in exophthalmic goitre, atrophy of ovaries and uteri (7 females), atrophy of seminal vesicles, prostate, and testes (3 males). The thyroid glands histologically showed an inactive colloid storage phase, the adrenals were large and active, the pituitary showed only relative atrophy. Group *2*, 12 rabbits received 2 mg. per kg. of 3,5-diiodotyrosine for 10 months. The animals of this group showed no muscle changes, and all females had enlargement of the ovaries and uteri similar to pseudopregnancy. Thyroid was only relatively inactive, and the pituitary showed no changes.—L. B. S.

THYMUS

Myelination in the central nervous system of the albino rat, treated with thymus extract (Hansen). Buckley, A., J. Comp. Neurol., 66:449. 1937.

The investigations of Rowntree, 'concerning the biological effects of thymus extract' on growth and development of the white rat, suggested a study of the central nervous system of treated animals from the standpoint of myelination. Spinal cords of thymus treated albino rats have been studied in comparison with normal standards by Watson, and with control animals of corresponding ages. The 6-day rat was selected as a starting point because in the normal there is well-defined myelination in the ventrolateral fasciculi, and the fasciculus cuneatus in the cervical region of the spinal cord, less marked in lower cord levels. The spinal cord of the sixth generation thymus treated rat shows myelination equivalent to that of the 13-day normal control, more advanced in the ventrolateral tracts than in the dorsal. In the tenth generation of 6-day treated animals, all of the tracts appear uniformly filled with myelinated fibers, except the pyramidal tract which normally contains few myelinated fibers in the adult animal. In this generation, myelination is equivalent to that in the normal rat 20 or more days old. To date, myelination has been found in all funiculi in treated albino rats of the tenth generation, at 48 hours and earlier; details to be given in later publication. Author's Abst., Wistar Bib. Service.